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Acute myeloid leukemias are a heterogeneous group of malignant diseases of hematopoietic progenitor cells with different clinical characteristics and variable outcomes with currently available treatments. In the last two decades, research has generated a rich and complex body of knowledge, revealing that leukemia, as well as other forms of cancer, is a disease involving several dynamic changes in the genome. Among these, mutations that produce oncogenes (with dominant gain of function) and tumor suppressor genes (with recessive loss of function) have been the first ones to be identified and have greatly contributed to dissect the biological and clinical complexity of AML. However, on this purpose new and very promising approach seems to be represented by genomics and post-genomics techniques like gene expression profiling and proteomics. Gene expression profiling relies on the fact that only a fraction of the genes in the genome is actively transcribed into messenger RNA (mRNA) in each type of cell and this expression of thousands of genes and to create a molecular profile of the RNA expressed in a given sample. Two approaches are generally used to classify a molecular profile of the RNA expressed in specific cell types. As with gene expression profiling, proteomics is useful for identifying proteins involved in various processes within the cells, although the analysis alone does not necessarily provide information regarding the cause or the function of the proteins expressed. However, proteomics, in some circumstances, also provides the possibility to study the physical interactions between proteins. Proteomics, thus, is useful for both large-scale surveys of proteins and detailed studies of the functional relationships among the proteins of interest. Both these approaches (gene expression profiling and proteomics) are expected to give a fundamental contribution to decipher completely the biologic and clinical diversity of AML. Examples of the set of informations so far provided by gene-expression profiling and by proteomics in AML will be presented.

MOLECULAR THERAPY OF AML: CLINICAL TRIALS WITH FLT3 INHIBITORS AND WITH IMATINIB

The clinical success of the specific tyrosine kinase inhibitor (TKI), Imatinib mesylate or ST1571 (1), has fostered onco-haematological research world wide to develop new molecularly targeted forms of therapy, particularly in AML. The targets of TKI are oncogenic tyrosine kinase of Class III receptor tyrosine kinase (RTK) family: the FLT3, KIT, FMS, and PDGF receptors. Particular interest has been aroused by the relatively high frequency of FLT3 receptor mutations found in AML. The FLT3 receptor has been found to be frequently targeted in AML and, to a lesser extent (at lower frequency), in myelodysplastic syndromes (MDS), by two different types of genetic alteration. Firstly, an internal tandem duplication (ITD) of the jM domain-coding sequence of the FLT3 gene (FLT3/ITD) is found in 20% to 41% of adult and pediatric patients with de novo or secondary
hypersensitivity, has been reported: all 43 AML patients received 145 Phase II trial in AML patients with SU5416 (Pharmacia) has been reported, and are still confidential. The MTD for phase II clinical trials in advanced malignancies was selected 50 mg po. Until now no result on efficacy has been reported on efficacy has been reported. Phase II trial in AML patients with CEP-701 or KT-5555(4-6) PKC412(7), CT53518, renamed as MLN518 (8) and SU11248 (9). Phase I trial in AML patients with MLN518 (Milennium) began at a dose of 50 mg po bid, increasing until the dose of 525 mg po pid. Toxicity was restricted to occasional nausea, vomiting and diarrhoea. In most patients these symptoms have been mild and manageable. CNS toxicity (weakness, tremor and clonus) has been also recorded. Until now no result on efficacy has been reported. Phase II trial in AML patients with PKC412 (Novartis) enrolled thirteen patients (median age 62, range 18-74, years) with relapsed or refractory AML, 10 pts having completed treatment. The initial starting dose was 40 mg po bid, increased to 60 mg po pid. After ex- vivo analysis showed incomplete inhibition of FLT3 auto-phosphorylation. Evidence of clinical activity was observed in 4 of 10 patients and correlate with consistent inhibition of FLT3. All 4 patients showed a decrease in PBL leukemic blast to <5% after 1 moths. Toxicity was mild and manageable. CNS toxicity (weakness, tremor and clonus) has also been recorded. Four AML FLT3 ITD or PM positive patients out of 50 has been treated with SU11248: all pts responded, obtaining transitory CR. Toxicity was mild at 50 mg, severe at 100 mg: grade I-II nausea was the main symptom, but weakness was the main toxicity. The TTD for phase II clinical trials in advanced malignancies was selected 50 mg po. Until now no result on efficacy in AML has been reported, and are still confidential. Phase II trial in AML patients with SU5416 (Pharmacia) has been reported: all 43 AML patients received 145 mg/m² i.v. one hour infusion twice weekly, via a central venous line. Treatment was well tolerated without nausea, headache and bone pain being the most frequent treatment side effects. One patient had a morphological remission lasting for two moths. Seven pts. achieved a partial response lasting 1 to 5 months. High VEGF m-RNA was the main constraint for predicting response. Phase II trials in AML patients with CEP-701 (Cephlon) and with TK787/ZK 222584, a specific inhibitor of both VEGF-receptor tyrosine kinases are ongoing. Phase I clinical trials that have been completed so far indicate, at least for PTK787/ZK 222584 is well tolerated in patients with advanced cancer. This work was supported by the Italian Association of Cancer Research (A.I.R.C.), by Italian C.N.R., by Cofin 2002 target projects on CML and by A.I.L. grants and Fondazione del Monte di Bologna and Ravenna and Ateneo 60% (Baccarani and Fiaccini).

References


Antibody-Targeted Therapy for Acute Myeloid Leukemia: Present and Future

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Treatment outcome for AML has substantially improved over the last decade, especially in younger patients who can tolerate intensified treatment strategies including hematopoietic stem cell transplantation. On the other hand, there has been little progress in the treatment of older patients where intensive chemothera-
apy regimens are associated with a lower CR rate, an increased risk of relapse and an inferior overall survival. The availability of antibodies reactive with antigens expressed only by hematopoietic cells has provided investigators with new tools for designing innovative treatment strategies for AML. The CD33 antigen is expressed on the surface of leukemic blasts in more than 90% of patients with AML. The antigen is also expressed by myeloid progenitors an to a lesser extent by mature myeloid cells, but not by primitive hematopoietic cells and non-hematopoietic tissues. Initial in vivo studies with an unmodified ('naked') murine anti-CD33 antibody in patients with advanced AML demonstrated that the MoAb was able to bind the leukemic cells both in the marrow and in the peripheral blood, and this was followed by a rapid internalization of the antigen-antibody complex. However, the antileukemic effect was modest consisting only of transient drops in the number of circulating blasts. A number of CD33-based immunotoxins have then been studied with encouraging results. The most promising of these novel compounds is Gemtuzumab Ozogamicin (GO, CMA-676, Mylotarg), a conjugate of a humanized anti-CD33 monoclonal antibody linked to the cytotoxic antibiotic calicheamicin, which as shown significant antileukemic activity in relapsed AML with a favorable side-effect profile. Phase II safety and efficacy studies have demonstrated that roughly 30% of patients with AML in first relapse achieve remission either with full recovery of platelet levels to normal (CR) or incomplete recovery of platelet levels (CRp) after treatment with 2 doses of GO at 9 mg/m², with toxicities including allergic reactions, prolonged myelosuppression, infections and a relatively high incidence of transient liver dysfunction resembling in occasional patients the picture of severe veno-occlusive disease. Other organ-specific toxicities, such as gastro-intestinal and mucosal, were mild or non-existent suggesting the potential for combination with standard chemotherapy. Such combination trials are currently underway both in previously untreated as well as in relapsed/refractory AML. One such trial focusing on GO as frontline treatment (with or without sequential chemotherapy) for newly diagnosed AML in elderly patients has recently been completed by the EORTC-LG in collaboration with GIMEMA. Results of this study as well as future strategies under development will be presented and discussed.
Indolent lymphomas are low-grade non-Hodgkin lymphomas (NHL) that may not require treatment for years. However, when therapy is needed, they are generally sensitive to chemotherapy but disease is difficult to eradicate completely and the residual clone eventually becomes resistant or transforms into a high-grade lymphoma. Despite recent advances in chemotherapy, radiotherapy and supportive care, overall survival for low-grade NHL has not significantly improved in the last decades. With the broad clinical impact of monoclonal antibodies (MoAb) against B-cell specific CD20 glycoprotein, immunotherapy has opened a new clinical era. While refining the use of unconjugated anti-CD20 MoAb either in combination with chemotherapy and immuno-modulatory agents or as single agent for maintenance therapy, conjugated anti-CD20 and new MoAbs targeting other surface B-cell antigens are under clinical investigation. Radioimmunoconjugates with Iodine-131 anti-CD20 or Itritum-90 anti-CD20 MoAbs have produced excellent results with overall responses up to 97% and low toxicity, but are clinically hampered by dose individualization. Among MoAb targeting CD22, humanised anti-CD22 RFB4 has shown CR in 60% follicular B-cell NHL (FL). Campath-1H, a humanized MoAb that targets CD52 on B and T cell lymphomas, shows poor efficacy on disease in lymph node and bone marrow and has found scarce application in FL, while in refractory chronic lymphocytic leukemia it produces 43% overall responses as a single agent and 63% in association with rituximab. Other MoAbs that have reached clinical evaluation include anti-CD80 and anti-HLA-DR Hu1D10 antibodies. Antisense approaches have also reappeared and oligonucleotides targeting bcl-2, which is overexpressed in most FL, and survivin molecules are being evaluated in clinical trials. G3139 (Genta Inc.) is the first antisense molecule widely tested in vitro and in vivo and synergistic enhancement in tumor cell killing has been found when combined with standard anticancer therapy. Fever, fatigue, hypotension and thrombocytopenia are the major side effects. Active vaccination strategies also seek larger clinical use. In particular, protein vaccines against the clonotypic tumor protein are clearly able to promote anti-tumor immunity and remove residual disease in the majority of FL patients having reached a clinical remission, but custom-made preparation of vaccines limits their broad clinical applicability. DNA vaccines encoding the very tumor specific Ig variable region determinants are easier to make and have also clearly shown anti-tumor activity against lymphoma models and a stage phase I/II dose escalation clinical trial for follicular lymphoma is under evaluation.

The experience with allogeneic hematopoietic stem cell transplantation (HSCT) in indolent lymphomas (NHL) and chronic lymphocytic leukemia (CLL) has been limited for the indolent nature of the disease, and because of the advanced age of the majority of patients. The more relevant advantages of allogeneic HSCT are the followings: 1) indolent NHL and CLL appears susceptible to the graft-versus-malignancy (GVM) effect, as demonstrated by the clinical response obtained after donor lymphocyte infusions (DLI); 2) the lack of tumor contamination of the graft. On the other hand the major disadvantages are the lack of suitable donors for the majority of patients and the high treatment-related mortality (TRM). The relevant TRM has confined this approach to young patients with refractory disease and/or extensive bone marrow involvement. Previous reports on myeloablative allogeneic transplantation have shown a low relapse rate but a high TRM. An IBMTR study on 113 patients reported a disease-free survival of 49% at 3 years with a 16% relapse rate, but unfortunately the TRM was 44%. The Verdonck group was the first to perform a study comparing allogeneic and autologous transplantation in patients with low-grade lymphomas: progression-free survival (PFS) was 68% versus 22% respectively at 2 years. Interestingly, both studies showed that allogeneic transplants could induce a sustained complete remission even in patients resistant or refractory to chemotherapy treatments. During last 5 years, reduced-intensity conditioning (RIC) regimens have been developed with the aim to reduce TRM while preserving the GVM effect. Although this RIC regimens vary considerably in the degree of immune-suppression and myelosuppression, they all induce allo-engraftment with a quite limited organ toxicity. Despite these improvements, GVHD remains a significant cause of mortality and morbidity and long-term results of RIC regimens are currently unknown. A recent EBMT report shows some promising results: the 1-year PFS rates were 61% and 56% in indolent lymphomas and CLL, respectively. We have previously reported the encouraging results of a pilot study of RIC in lymphomas: advanced stage patients could attain a durable clinical and molecular remission. An update of the GIMO protocol in 31 pts with indolent lymphomas and 16 with CLL shows that the estimated 2 year overall and progression-free survival were 77% (95%CI, 57-97%) and 78% (95%CI, 61-95%), respectively. So far a molecular marker has been identified in 15 patients in complete remission: 12 of 15 pts attained molecular remis-
sion in a time period going from 1 to 4 months after transplantation. In general, the TRM reported with RIC regimens ranges from 10% to 25%, indicating that the primary endpoint of reducing transplant toxicity has been probably accomplished. In conclusion, the evolving role of allogeneic HSCT in lymphoproliferative disorders is under active investigation and ongoing prospective trials will help to clarify which strategy is more appropriate for which subset of patients may have a durable benefit from the procedure.

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**MYELOPROLIFERATIVE SYNDROMES**

**(POLYCYTHEMIA, THROMBOCYTHEMIA, MYELOFIBROSIS)**

**POLYCYTHEMIA VERA**

Finazzi G

Divisione di Ematologia, Ospedali Riuniti di Bergamo, Italy

Polycthemia Vera (PV) is a chronic myeloproliferative disorder which results from clonal expansion of a transformed hemopoietic stem cell. Analyses of the pattern of erythroid and myeloid colony growth have demonstrated abnormal responses to several cytokines, raising the possibility of a defect in a signal transduction pathway shared by several growth factors. A number of cytogenetic and molecular approaches are now focused on defining the molecular lesion(s). Although there is no consistent cytogenetic abnormality detectable in PV, certain karyotypic changes, such as deletions of chromosome 20 or loss of heterozygosity on chromosome 9, are seen in a subset of patients. The evolution of PV, like that of most neoplasms, is likely to require several mutations accumulating in a single cell. Therefore, analysis of cytogenetic lesions evident in a subgroup of patients may identify genes whose activation or inactivation constitutes one of the multiple 'hits' necessary for disease development in these individuals. An exciting recent advance was the identification of molecular markers of PV. The first molecular marker described was a reduced expression of the thrombopoietin receptor c-Mpl on PV platelets and CD34-positive cells. Glycosilation and processing of c-Mpl is defective in PV cells and the extent of this deficiency correlates with disease duration and the degree of extramedullary hematopoiesis. Another molecular marker recently identified is PRV-1, a novel member of the uPAR receptor superfamily, which is overexpressed in granulocytes from patients with PV, but not detectable in granulocytes from normal individuals or patients with secondary erythrocytosis. Although PRV-1 is not expressed in resting granulocytes from normal controls, stimulation of these cells with granulocytes colony-stimulating factors induces PRV-1 expression. Several studies are ongoing to establish whether this novel hematopoietic receptor may play a role in the pathophysiology of PV or if it may be useful in the differential diagnosis of erythrocytosis. Although important advancements have been made over the last several years in the definition and understanding of the basic biological characteristics of the disease, thus contributing also innovative diagnostic criteria, therapeutic strategies have remained substantially unchanged. All patients should undergo phlebotomy with the goal of keeping the hematocrit value below 0.45. No additional therapy may be needed in
stable patients who are at low risk for thrombosis (age below 60 years, no history of thrombosis). In patients at high risk of thrombosis or who develop progressive thrombocytosis or splenomegaly, the choice of myelo-suppressive agent depends on the patient’s age. Older patients can be managed with radiophosphorus, low-dose busulfan or pipobroman. HU is the drug of choice in middle-aged patients despite concerns about its possible leukemogenicity. Interferon or anagrelide should be considered in younger patients, but their efficacy on hard clinical end-points, such as survival, major thrombosis and hematological progression remains to be demonstrated. In all patients with cerebrovascular, coronary or peripheral arterial ischemia, the association of low-dose aspirin is recommended. In addition, the results of the recently completed ECLAP trial will answer the question if aspirin should be given also to asymptomatic PV individuals for the primary prevention of vascular events. Innovative therapies, such as allogeneic bone marrow transplantation or imatinib mesylate have been successfully used in a few selected patients with PV but it is too early to define their role in the management strategy of disease.

ESSENTIAL THROMBOCYTHAEMIA: EPIDEMIOLOGICAL, DIAGNOSTIC AND THERAPEUTICAL ASPECTS
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The annual incidence of Essential Thrombocythaemia (ET) is around 18 per 1 million population and consequently about 1000 new diagnosis of ET are expected in Italy each year. Since no biological markers have been identified, the ET diagnosis is done on the basis of the updated PVSG criteria, by excluding the reactive thrombocytosis, the MDS and the other chronic MPDs. The diagnostic and prognostic value of the clonality demonstration has still to be defined. In a retrospective study of the Gruppo Italiano Malattie Mieloproliferative Croniche (GIMMC), in over two thousand patients, the overall survival in all age subgroups resulted to be shorter than in the matched Italian population, being the survival curves rather different after 9-10 years of follow-up. As expected the main causes of mortality were thrombosis (3.2%) and cancer (2.8%). Age over 60 years, previous thrombosis and uncontrolled thrombocytosis in the follow-up (identified by Cortelazzo et al. as high thrombotic risk factors), together with other new unfavourable prognostic factors (male gender, high platelet count and presence of peripheral granulocyte precursors at diagnosis) in the GIMMC study resulted to be associated with a significant decrease of patient survival. On behalf of the Italian Society of Haematology (SIE, SIES, GITMO) an Expert Panel has recently elaborated new evidence- and consensus-based practice guidelines for the therapy of ET. A platelet lowering treatment (target PLT below 400 x10^9/L; below 600 in case of toxicity or tolerability problems) has been strongly recommended in ET patients with: age over 60 years, or history of major thrombosis, or history of major haemorrhage, or platelet count over 1500x10^9/L; age of 40–60 years associated to a platelet count of 1000–1500x10^9/L and to a cardiovascular risk factor or a familial thrombophilia; age below 40 years and comorbidity with high thrombotic risk as homocistinuria or familial dominant hypercholesterolemia. The recommended first line therapy is based on the use of the non-mutagenic Interferons alpha and Anagrelide for the younger patients, and on the use of the cytotoxic Hydroxyurea, Pipobroman and Busulphan for the older people. Other recommendation are done for treatment of paediatric or pregnant patients and for treatment of thrombotic and haemorrhagic events. An antplatelet treatment, firstly with Aspirin 75–100 mg/day, is recommended in patients with symptoms or with cardiovascular risk factors, while it has to be avoided in patients with platelet count over 1500x10^9/L. Some more controlled studies are required to elaborate strong recommendation for treatment of ET, in particular for patients with intermediate platelet count (1000–1500x10^9/L) and/or with intermediate age (40–60 years).

MYELOFIBROSIS WITH MYELOID METAPLASIA
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Current knowledge about the pathogenesis of myelofibrosis with myeloid metaplasia (MMM) does not allow delineation of a single model that explains the proliferative advantage of the hematopoietic stem cells, the disruption of normal bone marrow extracellular texture with fibrosis, and extramedullary hematopoiesis. New concepts on the biology of the disease that could shed light on the nature of cellular proliferation abnormalities in MMM are related to abnormal CD34+ stem cell trafficking, neoangiogenesis and anomalous megakaryocyte proliferation. High number of hematopoietic stem cells mobilize and migrate from the bone marrow to the blood stream and colonize the spleen and other organs. Neoangiogenesis has now been documented as an integral component of medullary and extramedullary hematopoiesis. MMM platelets express a TPO-receptor (Mpl) isofrom that is incompletely glycosylated and poorly expressed on the cell surface and is linked to resistance to apoptosis and the aberrant signal transduction that provides a proliferative advantage. Experimental models in which mice given bone marrow grafts of cells infected with a retrovirus carrying TPO comple-
HEMATOPOIETIC STEM CELLS

HEMATOPOIETIC STEM CELL CHARACTERIZATION
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The highly orchestrated process of blood cell development and homeostasis is termed “hematopoiesis”. Understanding the biology of hematopoiesis and hematopoietic stem cells is essential for improving treatment of hematologic malignancies, congenital disorders, chemotherapy-related cytopenias, and blood and marrow transplants. Here we review the current state of the art regarding the definitions of hematopoietic stem cells (HSC), the methods of their isolation, their morphological and phenotypic characterization and their extensive self-renewal including their in vivo ability to rescue lethally damaged tissue in vivo for the lifetime of the recipient. The stem cell is a primitive cell that can divide either to reproduce itself (undergo self renewal) or to give rise to more specialized (differentiated) cells. It can undergo asymmetric self-renewing cell divisions, give rise to all blood elements, reconstitute the hematopoietic system when transplanted in lethally irradiated recipients, and engraft and differentiate in animals, even if the recipients is not irradiated. In humans, as in mice, the HSC can be purified to near homogeneity. Many systems have been used: a combination of approaches based on the physical and biological properties of stem cells and on the immunophenotype of the target cells. Density gradient separation is still commonly used. Pharmacologic cell cycle specific selection can be used as a pre-enrichment step in stem cell purification strategy. However, the most widespread methods for enrichment and purification use monoclonal antibodies directed against stem cell antigens (positive selection) or against antigens on differentiated hematopoietic cells (negative selection). Human HSC do not express lineage antigens but are CD45+. Unlike mouse HSC that do not express the CD34 antigen, human HSC are present within both the CD34+ and CD133+ cell fractions of bone marrow, blood, and umbilical cord blood. Furthermore HSCs express CD117, CD90 and CD164 antigens while they do not express CD38. In addition HSCs express a stem cell antigen defined by the recently described antibody W7C5. We and others recently demonstrated the presence of CD133+/CD34- cells in rhG-CSF mobilized peripheral blood stem cells. Cytofluorimetric analysis showed this sub-population constitutes 0.1-2% of the CD133-positive stem cells. Morphologically the CD133+/CD34- cells are fibroblast-like adherent cells which do not express...
HSC antigens. Kuci et al (Blood 101, 869,2003) reported these cells do not generate hematopoietic colonies in vitro. However, their transplantation into NOD/SCID mice induced substantially higher long-term multilineage engraftment rate than freshly isolated CD34+ cells suggesting the CD133+/CD34- cells are richer in SCID-repopulating cells. Xenografts transplants of HSC in NOD-SCID mice or fetal sheep require a minimum of 200-500 cells. It is not clear whether this is due to the xenotropic nature of the assay system or may reflect the lack or purity of HSC. An alternative approach based on the retroviral marking of HSC followed progeny of individual cells in vitro and in vivo showed that in both mice and humans single HSC can give rise to multiple progeny and undergo single HSC self-renewing cell divisions.

PLASTICITY OF HEMATOPOIETIC STEM CELLS
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The ability of various tissue-specific cells to develop into cells of unrelated tissue types has been reported by several investigators. This phenomenon, termed "stem cell plasticity" is particularly evident for bone marrow derived stem cells (both hematopoietic (HSC) and mesenchymal (MSC) stem cells). HSC can give rise not only to all the blood lineages but also to skeletal and cardiac muscle, endothelial cells, neuro-glial cells, lung, gut and skin epithelia, hepatic and biliary duct epithelium and pancreatic islets of Langerhans. This phenomenon has been reproduced occasionally "in vitro", consistently in animal models and observed in humans, particularly in HSC transplantation recipients. The possible mechanism underlying stem cell plasticity could include: 1) the presence of several types of tissue specific stem cells, 2) the persistence of true multi- or pluripotent stem cells in postnatal life, 3) the possibility of dedifferentiation of committed cells, 4) the fusion of donor cells with resident cells. In particular cell fusion, occurring spontaneously in osteoclasts, myotubes and cancer cells has been shown to account for the plasticity of HSC giving rise to hepatocytes but not to pancreatic beta cells. The definition of stem cell plasticity implies that 1) different cell lineages are derived from a single cell, 2) different cell types are functional in vitro and in vivo, 3) engraftment is significant and persistent. Demonstration of differentiation should rely on morphology, phenotype, functional tests, engraftment and rescue and until now very few studies have fulfilled all these criteria. However, plasticity of bone marrow derived stem cells is a real phenomena, whose biological and clinical relevance still requires carefully designed animal studies and pilot clinical trials.

EX-VIVO EXPANSION OF HEMATOPOIETIC STEM CELLS.
EXPERIMENTAL RESULTS AND CLINICAL USE
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Several clinical reports have validated the role of cord blood (CB) as an effective alternative source of hematopoietic stem cells (HSC) for allogeneic transplant. Indeed, 1) cryopreserved CB units are quickly available for patients lacking an HLA compatible HSC donor from family or from Bone Marrow Worldwide Donor Registry (BMWWDR), 2) a higher degree of HLA disparity between CB donor and recipient is acceptable and, finally, 3) the incidence and severity of GVHD are limited in recipients of CB transplant without any impairment of the graft-versus-leukemia effect. Nevertheless, in comparison with BM or peripheral blood (PB) the cellularity of CB units represents a major problem affecting clinical results of CB transplants: the rate of engraftment is decreased, the time to hematopoietic recovery is delayed and the wide use of this cell source in adults is limited. Therefore, in order to reduce the risk of transplant related mortality (TRM) associated with the prolonged post-transplant aplasia, several experimental studies for the amplification of the hematopoietic stem/progenitor cells have been investigated. The engraftment potential of the in vitro expanded cells has been evaluated in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice. However, the results of these studies still appear controversial. Aiming at the application to the human transplant setting, we have previously standardized an in vitro method for CB stem cell expansion designed on respect of clinical requirements. Therefore, CD34+ cells selected from thawed CB units were cultured on short-term in human AB or serum-free media in presence of three cytokines: FL, SCF, IL-3. In order to evaluate either the early hematopoietic reconstitution or the long-term engraftment potential, a NOD/SCID transplanted model was established. One-hundred mice were transplanted with amplified cells and the engraftment was compared to a control group of 57 mice receiving unexpanded CD34+ cells. The engraftment of human cells was evaluated in bone marrow (BM) and spleen samples by cytofluorimetric determination of the huCD45+ cells and by PCR analysis of human specific DNA sequences on hematopoietic colonies. In mice transplanted with amplified cells, a significant correlation between the rate of engraftment and the cell dose infused was observed (p=0.003). At the higher cell dose (7.5 x 104 input CD34+ cells), the rate of engraftment either in early (72%) or in long-term controls (90%) was com-

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parable with that of recipients of unexpanded cells (100%). As to concern the kinetic of engraftment in early controls (7-28 days after transplant), the amplified cells have an in vivo proliferative advantage on unexpanded cells. No significant difference in terms of number of human cells/colonies was detected in long-term controls. In an ongoing study, we are comparing the effect of multiple combinations of 4 cytokines (TPO, FL, SCF, IL-3) with the results previously obtained in short-term amplification. The combination of TPO/FL/SCF/IL-3 induced the highest amplification in terms of total nucleated cells (86 fold), CD34+ cells (8 fold), and hematopoietic progenitors (9.3 fold), whereas the lowest expansion was detected with the TPO/FL association (NC = 4.3 fold, CD34+ cells = 2.6 fold, CFC = 1.8 fold). However, the percentage of CD34+ cells (41%) and of the early CD34+/CD38- subpopulation (6.8%) obtained with TPO/FL was higher than after amplification with FL/SCF/IL-3 (8% and 0.54% respectively). These results show that the cells amplified in culture lacking IL-3 are more immature with a lower plating efficiency. The engraftment potential of the amplified cells was evaluated in NOD/SCID transplant. The preliminary results evidence that cells amplified with FL/TPO/SCF with or without IL-3 are the most effective in terms of either rate of engraftment (100%) or number of human CD45+ cells/progenitors detected in murine BM. The experimental results from in vitro studies and animal models show that different pre-clinical protocols for in vitro cord blood stem cell expansion might be designed in order to approach selectively the problems related to the hematopoietic reconstitution in CB transplant.

AUTOIMMUNE CYTOPENIAS

AUTOIMMUNE HEMOLYTIC ANEMIAS
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Autoimmune hemolytic anemias (AIHA) are a heterogeneous group of disorders characterized by the presence of autoantibodies directed against red cell surface antigens, and by a variable clinical picture and severity of hemolysis. AIHA can be distinguished in “warm” and “cold” based on the thermal properties of the antibody, and in primary (idiopathic) and secondary. Warm idiopathic forms are often insidious, although sometimes rapidly worsening. Life-threatening hemolytic episodes are more common in warm AIHA cases secondary to infections or in cold-antibody AIHA, which comprises idiopathic or secondary cold hemagglutinin disease (CHD) and paroxysmal cold hemoglobinuria (PCH). A positive Coombs’ test (direct antiglobulin test, DAT) is the cornerstone of diagnosis of AIHA. Anti-IgG antibodies are found in most cases of “warm” AIHA, being unusual to have isolated IgA or IgM, whereas cold agglutinins are virtually always IgM. More sensitive DAT techniques, including various immunoradiometric, ELISA and cytofluorimetric assays, the solid phase antiglobulin test, and the mitogen-stimulated DAT have been reported to give positive results in some patients with negative conventional DAT. Corticosteroids represent the first-line treatment of warm-antibody AIHA with overt hemolysis and are usually adequate to keep the hemolysis under control in 80-90% of cases. If the patient fails to respond, is intolerant or unable to achieve long-term remission on acceptable dosages, second-line treatment should be considered, and a choice has then to be made between splenectomy and immunosuppressive drugs (azathioprine, cyclophosphamide, and cyclosporin). The patient who has failed to respond to the first and second line treatment represents a critical clinical problem. Among possible remedies, besides blood transfusion, are high doses i.v. immunoglobulins, plasmapheresis (plasmaexchange or specific immuno-absorption of Ig), intravenous cyclophosphamide and vinca alkaloids. Other more recent options are Rituximab, Campath-1H, mycophenolate and bone marrow transplantation. Refractory and hyperacute cases with life-threatening immune-mediated hemolytic disease require often a therapeutic decision in emergency. CHD is usually a mild chronic hemolytic disease, often accompanying lymphoproliferative disorders. The acute transfert form of secondary CHD, usually associated with Mycoplasma pneumoniae or with infectious mononucleosis, is characterized by
rapid or abrupt onset, sometimes fulminant. The direct Coombs test is positive against C3d, and negative against IgG. The specificity of cold agglutinins is anti-I, anti-i, or anti-Pr. Patients with mild chronic hemolysis do not require specific therapy, but avoid cold as far as it is practicable. In patients with severe symptoms treatment options include chlorambucil, cyclophosphamide, and more recently Rituximab, with very promising results. Corticosteroids and splenectomy are almost always ineffective. PCH was classically described in patients with syphilis, but is now frequently associated with viral infections. There are chronic and acute, transient forms, in which the resultant anemia can be very severe. Recovery from the attack usually is rapid. The antibody causative of PCH is the Donath-Landsteiner "biphasic" hemolysin, a complement-binding IgG that binds to red cells at 4°C, and becomes hemolytic after incubation with complement at 37°C. The specificity of antibody is almost invariably anti-P, with few exceptions. In conclusion, hemolytic anemias are a heterogeneous group of disorders with a variable degree of severity, and consequently require different therapeutic decisions. Refractory and hyperacute cases represent a critical clinical problem. New promising therapeutic options are now available, but need better validation by appropriate clinical trials.

AUTOIMMUNE THROMBOCYTOPENIAS
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Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by a persistently reduced platelet count (Plts <150x10^9/L) due to the premature platelet destruction caused by autoantibodies binding to platelet membrane glycoproteins. In most of the cases, diagnosis of ITP remains one of exclusion, although case-history, physical patient’s examination, peripheral blood smear observation are the pivotal components of diagnosis. Bone marrow examination is mandatory in all cases where treatment is requested and in patients aged more than 60 years because of the risk of myelodysplasia misdiagnosis. Disease clinical pattern is generally chronic in adults and acute in children. Bleeding symptoms are more frequent in children than in adults. Therapeutic approach of ITP is based on the evaluation of bleeding symptoms and of platelet levels at diagnosis. Treatment is mandatory if platelet levels are below 20-30x10^9/L, which are considered safe, or bleeding symptoms are present. In adults, prednisone is usually the first-line therapy, at daily induction doses about 1 mg/kg. Complete, partial or minimal responses are obtained in about 60-70% of the cases; no responding patients (30-40%), after 6-8 months follow-up, are candidate to second-line therapy. In elderly patients (aged > 65-70 years) prednisone, at lower daily doses (0.25-0.5 mg/kg), is the first-line therapy, but, in some cases, HDIg or Ig anti-D could be also used. Response rate is similar to that of younger patients. In young adult resistant to or relapsing after prednisone therapy, with bleeding symptoms or platelet levels < 20x10^9/L, second-line therapy is represented by splenectomy. In more aged people, splenectomy is not indicated and other immunosuppressive drugs (such as azathioprine) can be proposed. In children candidate to therapy, prednisone at intermediate-high doses or HDIg are the main therapeutic proposals. Response rate is generally very high (> 80%) and it tends to persist in the time. Second-line therapy in children aged more than 6-7 years is splenectomy: this approach could be reserve to very high-risk patients, in whom low platelet levels and haemorrhagic symptoms persist despite therapy. However, in some cases, periodic infusions of HDIg can delay splenectomy, by achieving persistent disease remission. Patients (children and adults) who do not respond to second-line therapies are considered affected by “refractory ITP” and for them, experimental approaches are sometimes necessary. ITP patients lacking criteria for beginning therapy, must be observed during long term follow-up with the aim to evaluate possible spontaneous remission or need for therapy start. Moreover, observation during the time, allows to identify the appearance of other pathologies.

AUTOIMMUNE NEUTROPENIA
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Neutropenia is defined as an absolute neutrophil count (ANC) of less than 1500/mL. Usually it is defined as mild when the ANC is between 1000 and 1500/mL, moderate between 500 and 1000/mL and severe with ANC less than 500/mL. The risk of infection, that is the only significant consequence of neutropenia, begins to increase at an ANC below 1000/mL. Neutropenias can be classified as acquired or congenital. While the latter are rare conditions, the former are frequent and recognize many causes, with infections, drugs and immune disorders being the most common. Antineutrophil antibodies (Abs) are well recognized causes of neutropenia. They mediate neutrophil destruction either by splenic sequestration of opsonized cells or by complement-mediated neutrophil lysis. Several tests are available for detecting antineutrophil Abs including agglutination, direct and indirect immunofluorescence, direct and indirect antinuclear assays. Direct methods detect Abs on a patient’s neutrophils but they are hampered by a possible nonspecific binding of immune complexes to Fc receptors.
expressed by neutrophils. The indirect methods test the patient’s plasma or serum against normal cells and they are considered to be the best Abs screening procedure. Different types of immune-mediated neutropenia have been identified. Isoimmune neonatal neutropenia is a self-limiting condition due to the transplacental passage of IgG Abs to neutrophil-specific antigens (Ags) inherited from the father; the pathogenesis of this disorder is identical to that of Rh hemolytic disease. Anti-neutrophil Abs are frequently found in recipients of repeated granulocyte transfusions. In these cases, as in isooimmune neutropenia, Abs are directed against HLA Ags or against Ags specific for neutrophils. The true autoimmune neutropenia (AIN) is a rare disorder that can be either primary or secondary. In primary AIN, mostly seen in young children, neutropenia is the sole abnormality and, although neutrophil counts are often below 500/mL, it is rarely associated with serious infections and shows a self-limited course. In this condition neutrophil-specific Ab frequently recognized NA1 or NA2 Ags located on the IgG-Fc receptor type 3b (FcγRIIb or CD16). In few patients autoAb with CD11a or CD11b specificity were detected. Secondary AIN are more commonly seen in adults and underlying causes include collagen disorders, drugs, viruses and lymphoproliferative disorders of B- and T-cells types. In secondary AIN autoAbs tend to be directed at the total FcγRIIb rather than its subunits and the resulting severity of neutropenia may be greater with more severe infectious complications. CD8-positive T-cell-mediated inhibition of granulopoiesis in the bone marrow has been described in AIN secondary to rheumatoid disease and myelodysplasia. G-CSF is a proven treatment in patients with AIN of all types and is now preferred to other possible therapies such as high-dose intravenous immune globulins, corticosteroids, cyclosporin and danazol. A different type of immune-mediated neutropenia is the pure white cell aplasia, a rare disorder characterized by complete disappearance of granulocytopenic tissue from the bone marrow. It is often associated with thymoma and is due to the presence of Ab-mediated GM-CFU inhibitory activity.
es with documented resistant Gram-positive infections that do not clinically respond to initial empirical therapy. In conclusion, a correct estimate of the risk of infection nowadays allows a strategy of differentiated empirical therapy in the management of cancer patients with febrile neutropenia.

**NEW ANTIFUNGAL AGENTS: A REAL PROGRESS IN THE MANAGEMENT OF INVASIVE MYCOSES?**

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In the last years, considerable progresses have been achieved in the management of invasive mycoses in immunocompromised patients. However, the prognosis of these infections, in particular those caused by filamentous fungi, continues to be dramatically poor with mortality rates reaching more than 80% in selected categories of patients. New antifungal drugs, among the classes of triazoles and echinocandins, with interesting antimicrobial and pharmacokinetic characteristics, are under investigation and important trials have been reported in the last two years. These studies seem to suggest the relevant role of these new drugs in the antifungal armamentarium. The triazole voriconazole and the echinocandin caspofungin demonstrated significant advantages in terms of toxicity, and response rate, when compared to conventional amphotericin B in the treatment of aspergillosis and candidiasis, respectively. Other non controlled studies showed a promising role of caspofungin in the treatment of Aspergillus infections and of voriconazole in the treatment of other rare mycoses. These experiences with new antifungal drugs seem to show exciting perspectives in the therapeutic strategies of life-threatening mycoses and seem to indicate that amphotericin B no longer is the “golden standard” for a variety of fungal infections. However, due to the peculiar characteristics of the design of these studies, the interpretation of the results may be difficult and further confirmatory experiences are needed. In conclusion, despite the promising results of the recent experiences in the management of invasive fungal infections, last generation drugs need to be further investigated in proper trials and old drugs, first of all conventional amphotericin B, continue to have a central role in the antifungal armamentarium.

**CYTOKINE RECEPTOR SIGNALING IN ACUTE MYELOID LEUKEMIA**

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Numerous genetic abnormalities have been identified in acute myeloid leukemia (AML). Alterations in transcription factors due to chromosomal translocations have been observed in particular morphological subtypes of AML. In addition alterations in molecules that normally regulate cell behaviour in response to external cues (cytokines) are common in AML, but these types of mutations are less closely associated with particular morphologies. The first type of alteration play a major role in the block of cell differentiation observed in leukemic cells, while the second alterations are involved in the proliferation and survival advantage of leukemic cells. The cooperation between these two types of alterations is required for the development of the leukemia. Several alterations of cytokine receptors have been observed in AML. Among them we can distinguish between qualitative and quantitative abnormalities. The most frequent qualitative abnormality of cytokine receptors observed in AML consists in various types of alterations observed at the level of Flt3, a class III receptor tyrosine kinase; these mutations lead to the presence of a constitutively activated receptor, whose anomalous signalling leads to a constitutive activation of Stat5. In other cases Flt3 is simply overexpressed in the absence of any mutation. The presence of a mutated or hyperexpressed Flt3 is associated with a poor prognosis. The most frequent abnormality is represented by an overexpression of the IL-3Ra, observed in about 40% of the AML patients and associated with an high cycling activity, increased resistance to apoptosis triggered by growth factor deprivation, spontaneous Stat5 activation. At the clinical level the IL-3Ra overexpression was associated with high blast cell counts at diagnosis, and lower remission rate. Deregulated expression of IL-3Ra may contribute to the proliferative advantage of leukemic blasts and, hence, to a poor prognosis. Increased angiogenesis is shown in bone marrow biopsies of patients at diagnosis of AML. Furthermore, VEGF levels appeared to be of prognostic value in newly diagnosed cases of AML and VEGFRs (I, II and III) are often expressed on AML blasts. The analysis of VEGFR-I expression in AML blasts showed a pattern of expression preferentially associated with some leukemia sub-
types and with the presence of specific molecular abnormalities (i.e., the presence of a mutated Flt3). The VEGFR-III showed a pattern of expression similar to that observed for the VEGFR-1, with the only exception that it was more frequently expressed in leukemias with granulocytic features. The expression of VEGFRs on AML blasts seems to be associated with a high blast cell counts and could represent a negative prognostic factor. The presence of abnormalities of cytokine receptor signalling in AML has shown that they may represent a therapeutic target. Several inhibitors of cytokine receptor signalling have been discovered and they are the objective of intensive experimental and clinical studies.

MOLECULAR MECHANISMS OF VESSEL FORMATION
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To distribute nutrients throughout the body vertebrates have evolved a hierarchical branching blood vascular system that terminates in a network of size-invariant units, namely capillaries. Development of capillary networks characterized by typical intercapillary distances ranging from 50 to 300 µm is instrumental for optimal metabolic exchange. The ability to form networking capillary tubes is a cell autonomous property of the endothelial cells (ECs), which do need permissive but not instructive signals from the extracellular environment. In the embryo, the morphogenetic process by which free EC precursors (angioblasts) of mesodermal origin self-assembly into a primitive vascular plexus is known as vasculogenesis. Angioblasts either coalesce at the location where they emerge from the mesoderm (type I vasculogenesis) or they migrate through tissues and form blood vessels at distant sites (type II vasculogenesis). Angiogenesis is the cellular mechanism by which the primitive embryonic vasculature is remodeled into a mature vascular bed comprising arteries, capillary networks, and veins. Angiogenesis also includes penetration by sprouting and branching of vessels into avascular regions of the embryo, such as ectodermal or mesenchymal tissues. Besides to sprouting angiogenesis, intussusceptive angiogenesis is a relatively new concept in vascular biology and consists in the repeated insertion of new slender transcapillary tissue pillars, which subsequently increase in size, thus allowing the capillary network to grow in itself. After birth, angiogenesis occurs in physiologic situations such as endometrial proliferation, wound healing, and organ hypertrophy. An operating and stable vascular bed results from a balance of signals that favour angiogenesis and vascular stabilization, and those that promote vascular regression; deregulated angiogenesis is pivotal in tumor progression, inflammatory and viral diseases, retinopathies and vascularization of ischemic tissues. The latter is also characterized by arteriogenesis and allows the transformation of a small arteriole into much larger conductance artery. Small arterioles that interconnect side branches proximal from the arterial occlusion with distal ones experience increased fluid shear stress. This activates the endothelium and leads to monocyte adhesion and infiltration with the subsequent production of growth factors and proteases. Sprouting angiogenesis it is an enough known process occurring in stages, which orchestrate a network of cooperative interactions. They include 1) an initiation phase, characterized by increased vasopermeability, 2) the progression constituted by the production of proteolytic enzymes, that degrade the extracellular matrix and favour endothelial cell migration, and the entry of cells into either cell-cycle or apoptosis response, 3) the differentiation into new vessels, 4) the stabilization and maturation of vessels by mediator molecules that recruit mesenchymal cells to vessel walls, and 5)and the vessel guidance phase which defines the final trajectory of the nascent vessel. Endothelial cells, pericytes, smooth muscle cells and bone marrow-derived precursors are the cell types that form the vascular unit. Their reciprocal interactions are regulated and stimulated by specific growth and differentiating factors (VEGFs, angiopoietins, Semaphorins), physical forces, proteins of extracellular matrix and proteolytic enzymes. These may represent putative targets for a pharmacological intervention to inhibit or favour angiogenesis in different pathological settings.

NEW ADVANCES IN ANTIPHOSPHOLIPID SYNDROME
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Antiphospholipid antibodies are a wide and heterogeneous group of immunoglobulins that include, among the others, lupus anticoagulants and anticardiolipin antibodies. The interest towards them comes from their association with (recurrent) arterial and venous thrombosis, and obstetrical complications in the antiphospholipid syndrome. Lupus anticoagulants are acquired inhibitors of coagulation, which prolong the phospholipid-dependent coagulation reactions, and anticardiolipin antibodies react with anionic phospholipid in solid phase immunoassays. In the 1990s, work from different laboratories made it clear that lupus anticoagulants and anticardiolipin antibodies do not recognize anionic phospholipids, as long believed, but plasma proteins bound to suitable anionic surfaces. b2-glycoprotein I, and prothrombin are the most common and investigated antigens. (Activated) protein C, protein S, oxidized low-density lipoproteins, annexin V, high- and...
low-molecular weight kininogens, factor XII, thrombomodulin, tissue-type plasminogen activator, factor VII/VIIa, complement components C4 and H are other antigenic targets of antiphospholipid antibodies. Because most of these proteins are involved in the initiation and control of blood coagulation, it is conceivable that antibodies that reduce their availability or hamper their function may affect the pro- and anticoagulant balance. This might represent the pathophysiologic background underlying the increased thrombotic risk of antiphospholipid-positive patients. Interference with the protein C anticoagulant pathway has been reported for lupus anticoagulants and antib2-glycoprotein I antibodies both in plasma and in vitro systems. Recently, our group showed that also antiprophthrombin antibodies hamper the inactivation of factor Va by the protein C pathway in an in vitro system. Furthermore, some antiphospholipid antibodies have been reported to displace the annexin V shield from procoagulant surfaces, induce monocyte tissue factor, recognize, damage, and/or activate endothelial cells, induce platelet agglutination or aggregation, and interfere with antithrombin activity and the fibrinolytic pathway. However, despite a wealth of research, no definite and convincing demonstration of a pathogenic role of antiphospholipid antibodies in the development of thrombosis in the syndrome has yet been given in humans. Recently, we performed a systematic review of the antiphospholipid syndrome to investigate the association between thrombosis and some antiphospholipid antibodies. With respect to lupus anticoagulants and anticardiolipin antibodies, we focused on 25 prospective, cross-sectional, ambispective and case-control studies, on more than 7,000 patients and controls: lupus anticoagulants were a clear risk factor for thrombosis, irrespective of the site (arterial or venous) and type (first event or recurrence) of thrombosis, the presence of systemic lupus erythematosus, and the methods used to detect the antibodies. Anticardiolipin antibodies were not such a strong risk factor, unless the G isotype, medium or high titers, and arterial thrombosis were considered. Thirty-two, mainly retrospective, studies on more than 7,000 patients and controls allowed to investigate the association of antib2-glycoprotein I antibodies and thrombosis: 57% of the associations reached statistical significance for antib2-glycoprotein I antibodies, and only 37% in the case of antiprophthrombin antibodies. In conclusion, the relationship between most antiphospholipid antibodies and thrombosis requires to be further clarified by clinical and laboratory studies.

Peripheral Blood Progenitor Cell (PBPC) are widely employed in autotransplant procedures. The extent of PBPC mobilization and harvest is variable, depending on several factors, including type of mobilization schedule, disease characteristics, previous treatments. In order to further investigate factors influencing the extent of PBPC mobilization, data were collected from 1,907 procedures performed by 49 Centers associated to the GITMO in the period January 1, 1999–January 1, 2000. Patients had a median age of 47 yrs. and their diagnosis was: acute myeloblastic leukemia (AML) (226 pts., 11.8%), acute lymphoblastic leukemia (76 pts., 4%), non-Hodgkin's lymphoma (572 pts., 30%), Hodgkin's Lymphoma (44 pts., 2.2%), solid tumor (424 pts., 22.2%), non-malignant disease (80 pts., 4.2%). The mobilizing treatment was part of the front-line induction therapy in 1,336 pts. (70.4%), whereas 560 pts. (29.3%) underwent mobilization for refractory or relapsed disease. Mobilization was induced by G-CSF + chemotherapy at high-dose (hd) in 1,078 pts. (56.5%) or at conventional dose in 609 pts. (31.9%), or by G-CSF alone in 206 pts. (10.8%) (other procedures: 0.7%). When combined with chemotherapy, G-CSF was employed at 5 µg/kg/day in 1,277 pts. (76%) or at higher doses in 405 pts. (24%). According to the total amount of collected CD34+ve cells, we identified two main groups of patients, i.e.: Poor Mobilizers (P.M.), collecting <2x10^6 CD34+ve cells/kg b.w. (366 pts., 19.2%) and Good Mobilizers (G.M.), collecting ≥2x10^6 CD34+ve cells/kg b.w. (1,541 pts., 80.8%). The following aspects were found to affect the extent of PBPC mobilization: i. disease status at mobilization (28% P.M. in refractory/relapsed pts. vs. 15% P.M. in pts. treated front-line); ii. disease type (34% P.M. in AML at induction vs. 11% P.M. in all other diseases); iii. mobilization schedule (25% P.M. with G-CSF alone vs. 18% P.M. with chemotherapy + G-CSF); iv. peripheral blood counts at the mobilizing treatment (39% and 29% P.M. if WBC were <4000 and platelets were <150,000, respectively, vs. 16% P.M. with higher blood counts). In multivariate analysis, relapsed/refractory disease, AML, WBC <4,000 and mobilization without chemotherapy were found to be independently associated with reduced PBPC mobilization. On the contrary, PBPC mobilization was not significantly influenced by dose of chemother-
apy, dose of G-CSF, disease other than AM L and bone marrow involvement. In conclusion, the analysis of 1,907 procedures allowed to define: i. type of disease more often considered for PBPC-based autografting programs; ii. mobilization procedures commonly employed by the GITMO Centers; iii. factors that have the highest influence on the extent of PBPC mobilization; iv. parameters reliably predictive of the amount of PBPC that will be collected.

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TREATMENT OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN SECOND REMISSION: RISK OF A FURTHER RELAPSE DURING THE SEARCH OF AN UNRELATED DONOR

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Despite the progress that has been made over the last twenty years in the treatment of childhood acute lymphoblastic leukemia (ALL), patients who experience a relapse still have dismal prognosis when treated with conventional chemotherapy. Stem cell transplantation (SCT) from a matched related donor (MRD) cures more than 50% of patients who failed first line chemotherapy, whereas indications for autologous transplantation are limited to a small subset of patients with either late bone marrow relapse or extramedullary recurrence. Results recently reported by the AIEOP (Associazione Italiana di Ematologia ed Oncologia Pediatrica) show that the probability of 2 year DFS of children given bone marrow transplantation (BMT) from an unrelated donor (UD) is similar to the probability offered by SCT from MRD. This improvement is mainly due to refinements in HLA-typing, GVHD prophylaxis, timing of SCT, and supportive care. However, HLA polymorphism is still a major obstacle in finding a fully matched UD for all patients lacking a MRD, despite the availability of more than 7 million volunteer UDs in world-wide registries. Because of this, several transplant centers have recently offered transplantation from alternative donors, either HLA-mismatched relatives, or unrelated umbilical cord blood (CB) donors. Thanks to the creation of CB banks, more than 80,000 CB units have been made available for transplantation allowing more than 2,000 unrelated umbilical cord blood (UCBT), and 28% of 2-year DFS of children with ALL who underwent UD CBT reported by the Eurocord Registry is not different from the percentage reported in children undergoing UD BMT. That is why it has been suggested that UD CBT should be taken into consideration for all children with poor prognosis ALL who are eligible for an UD transplant. Results obtained in children with ALL given haploidentical SCT are less clear. In a retrospective study of the AIEOP, we have recently analyzed a cohort of 167 consecutive children with second remission ALL after a first marrow relapse, for whom an unrelated bone marrow donor search was activated between 1989 and 1998. A suitable donor was identified for 70 patients at a median interval of 5.4 months (range 1.6-15.6) from search activation but leukemia relapse occurred during the search in 94 children (56%) at a median interval of 4 months from search activation (range 10 days-56 months). At 6 months, the actuarial probability of finding a donor and of leukemia relapse was 33.4 and 38.1%, respectively, and the 6 months probability of finding a donor before relapse was 19.5%. Overall, there were therefore more relapses than identified donors. We concluded that a relapse is the major limiting factor for UD BMT in children with ALL in second CR after a bone marrow relapse (b), it is important to perform it or other types of SCT within 4 months from relapse. In order to evaluate the impact of different therapeutic approaches on patients with leukemia candidates for SCT, the AL Working Party (WP), the Pediatric Diseases WP and the Immunobiology WP of the European Bone Marrow Transplant Group decided to conduct a prospective, multicenter, non randomized trial.

TRANSPORT STRATEGIES FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA LACKING A HLA COMPATIBLE SIBLING

Frassoni F, Labopin M, Rocha V, Ibatici A, Bacigalupo A, Martelli M, Gluckman E, on behalf of Eurocord and Acute Leukemia Working party EBMT

Hematopoietic cell transplantation (HSCT) is a well established therapy for patients with (AML). There is consensus that HSCT is the therapy associated with lower incidence of relapse. However, not all experts agree that if an HLA compatible sibling is available the patient should undergo HSCT. Thus, for patients older than 35-40 yrs several authors consider that chemotherapy is associated with a 5 yrs survival which is not inferior to HSCT. In this abstract we shall not discuss this issue but we shall remain restricted to transplant options. In fact, other experts think that HSCT is the best approach. This latter approach is accepted in all quarters for high risk patients or patients not in first remission. Moreover, while the use of HSCT as consolidation therapy remains disputable, in absence of an HLA identical siblings there is no agreement on the type of HSCT approach. We have analysed the results of HSCT using different sources of hematopoietic stem cells (HSC) such as: 1) Unrelated Donor, 2) Umbilical Cord Blood, 3) Haplo-identical relatives, 4) autografting. Using Eurocord and ALWP-EBMT registry data a matched pair analysis was performed in order to compare the results of UD-UCBT versus unrelated bone marrow transplants (UBMT) in adults with acute leukemia (AL). Eighty seven adults (>16 years) with AL receiving UD-UCBT from 01/98 to 01/2002 were
matched for age, diagnosis, status of disease at transplant and use of total body irradiation with 174 non T cell-depleted UBM T performed in the same period. The median follow-up time of UCBT and UBM T were 16 months (2.5-42) and 11 months (0.8-42) respectively. For the whole group of patients (n=261) the median age was 25 years, 46% were transplanted for AM L and 54% for ALL, in first CR (24%), second CR (21%) and more advanced disease (55%). The main statistically differences (p<0.001) between the groups were: 1) HLA compatibility (defined as HLA-A, -B by serology and DRB1 allelic typing); all UBM T were HLA matched whereas UCB grafts were mismatched in 90% of patients (1 HLA diff=49%, 2 HLA diff=37% and 3 HLA diff=4%); 2) cord blood graft contained 10 times less nucleated cells per recipient's kg and 3) GVHD prophylaxis consisted mainly in CsA+Pred in UCBT (73%) and CsA+MTX (88%) in UBM T. Results. Neutrophil recovery (>500/µL) was delayed after UD-UCBT. Median time to neutrophil recovery was 28 days after UCBT compared to 19 days after UBM T (<0.001). Incidence of acute GVHD (≥II) was 32±5% after UD-UCBT compared to 41±4% after UBM T (p=0.05) and 2 yr chronic GVHD was not statistically different (p=0.53). Kaplan-Meier estimate of transplant related mortality at day 100 and 2-years were respectively 37±5% and 66±5% after UD-UCBT compared to 27±3% (p=0.08) and 46±5% (p=0.12) after UBM T. 2 yr-relapse incidence was similar in both group of patients (45±9% after UCBT versus 44±6%, p=0.94). Finally, 2 years probability of overall survival (OS) was 31±5% after UD-UCBT and 34±3% after UBM T (p=0.25) and leukemia free survival (LFS) was 24±5% and 30±4% (p=0.21), respectively. In conclusion, these data suggest that despite increased HLA disparities, probabilities of relapse, OS and LFS after UD-UCBT are comparable to those observed after UBM T. Therefore UD-UCBT with high number of cells infused (>1.0 x10^7/kg) and no more than 2 HLA disparities should be considered an acceptable alternative to HLA-matched UBM T for adults with AL. In a recent study including UBM T for acute leukemia, at 4.5 years patients with KIR ligand incompatibility had higher probability of overall survival (87% vs. 48%, p=0.006) and disease-free survival (87% vs. 39%, p=0.0007) compared with those without KIR ligand incompatibility. Transplant-related mortality for the two groups equaled 6% and 40% (p=0.01), respectively. Relapse rates for patients transplanted from a donor with or without KIR ligand incompatibility were 6% and 21%, respectively (p=0.07). All patients with myeloid malignancies transplanted from KIR ligand disparate donors (n=13) are alive and disease-free. These data indicate that NK cell alloreactivity is associated with better outcome after URD-HSCT when ATG is used as part of GVHD prophylaxis. Autograft option. Several studies have shown that autograft may be superior to chemotherapy. However in the MRC study, 330 randomised patients showed that there is no advantage in using an autograft as Course 4 or 5 (i.e. instead of chemotherapy). Careful analysis of other trials demonstrates that autograft is not superior or with respect to survival, either overall or within any risk group. It does however reduce the risk of relapse in all subgroups. This is confirmed in a meta-analysis. The EBMT analysis concerning patients (n=2171) with AML 1CR transplanted from 1/1/96 to 31/1/2001 show the following results. Median age 44(18-60); in 22% of patients the conditioning included TBI; 95% of patients received an unpurged autograft; source of stem cells was peripheral blood in 68% of patients; interval CR-BMT 113 days(21-705). The 5 yrs LFS, OS, RI, TRM was: 43%±2, 51%±1; 53%±1; 9±1. Although a more detailed analysis would identify different outcomes among prognostic groups, and considering that this may represent a selected population, the results indicate that autografting in AML in 1CR is an important option for patients who lack HLA compatible siblings. Haplo-identical transplant has been leaded by the Perugia group. Thus, we report their results from the most recent publications. 33 AML patients were transplanted with median age 38 (9-62) yrs. All were at high risk because of relapse at transplant, or second or later CR or CR1 but with unfavourable prognostic features. A positive selection of the CD34+ cells was used. TBI-Fludarabine-based conditioning and no post-transplant immunosuppressive therapy was given. Leukemia relapse was largely controlled in AML recipients whose donor was NK alloreactive, with only 2 out of 16 relapsing. To date, 13 of 18 AML (72%) who were in any CR at transplant, survive disease-free while 4 of the 15 patients (27%) in relapse at transplant survive. The probability of LFS for patients transplanted in CR was 60% and was significantly better in the 16 AM L patients whose transplant included donor vs recipient NK cell alloreactivity (70% vs 7%). In conclusion, for patients with AM L lacking an HLA compatible sibling it remains difficult to make any recommendation but several options are available. However, a registration study is needed to evaluate which is the best transplant choice taking into account the urgency to proceed to HSCT in relation to diseases status and clinical conditions and the time needed to organise the transplant.
chromosome 12p deletions occur with a 3-5% incidence in adults with acute lymphoblastic leukemia (ALL) as assessed by cytogenetic analysis. Usually the 12p13 band, where the ETV6 gene is located, is involved. To better define the incidence and significance of 12p/ETV6 deletion in adult ALL, 223 patients were studied by interphase FISH using a 12p13/ETV6 probe and an AML1 probe as control. The majority of patients were enrolled in the 0496 /ALL2000 GIMEMA treatment protocols and were submitted to cytogenetic and molecular investigation for the presence of BCR/ABL fusion, MLL rearrangement, PBX/E2A fusion, and TEL/AML1 fusion. Using a hierarchical cytogenetic/molecular classification, 73 patients had the t(9;22)-BCR/ABL fusion; 28 had t(4;11)-MLL rearrangement or 6q- or t(1;19); 19 had a pseudodiploid karyotype; 10 had a hypodiploid karyotype; 19 had a hyperdiploid karyotype; 51 had a normal karyotype and 23 had a hypodiploid karyotype. The karyotype in these patients was as follows: abnormal 12p in 3 cases; t(9;22) in 3 cases; 1 hypodiploid; 2 normal; 1 no mitoses. Eight out of the ten patients with 12p deletion displayed a B-immunophenotype (pre-B in 7 cases, pro-B in 1 case), while both cases were classified as T-lineage ALL. The age of the patients ranged between 16 and 60 years with a median age of 42; the WBC count at presentation ranged between 3850 and 314,000/mL (median 15900). A complete remission was achieved in 7/10 patients, three of whom relapsed after 2, 8 and 20 months; four are alive in 1st CR at 7-47 months. Four patients died after 1-24 months and the median overall survival was 23.9 months. We arrived at the following conclusions: a) the 12p/ETV6 deletion is the fourth most frequent anomaly occurring in adult ALL after t(9;22), 11q23 rearrangements and 6q-; b) with the exception of t(9;22), it is not found in association with other classical anomalies (11q23 rearrangements, 6q-); c) it is usually associated with a pre-B immunophenotype. Though the number of cases is small, the outcome in our patients appeared to be severe, even when excluding patients with t(9;22) from the analysis.
patients with similar disease characteristics with MEL100 or MEL200 and compared their toxicities and outcomes. Ninety previously untreated myeloma patients were treated with two MEL100 courses. Their clinical outcome was compared with a control group of 90 pair mates matched for serum xβ-2 microglobulin levels and Durie and Salmon clinical stage, treated at diagnosis with two MEL200 courses. Age was significantly different between the two groups (p<0.001), no differences in other clinical characteristics were observed. Transplant-related mortality was 4% after MEL100 and 5% after MEL200 (p=NS). Complete remission (CR) was 35% after MEL100, 48% after MEL200 (p=0.08). Median event-free survival (EFS) was 32 months in the MEL100 group, 42 months in the MEL200 group (p<0.005), but overall survival (OS) was 67 months for MEL100 and 75 months for MEL200 (p NS). After MEL100, the duration of hospitalisation (p=0.05), of severe neutropenia (p<0.001) and of thrombocytopenia (p<0.001), transfusion requirements (p<0.002), incidence of mucositis (p=0.016) and fever of unknown origin (p=0.008) were all significantly reduced. MEL100 conditioning regimen was significantly less toxic compared with MEL200. MEL100 was similar to MEL200 in terms of CR rate and OS, while EFS was significantly increased (p=0.008). Median event-free survival (EFS) was 32 months in the MEL100 group, 42 months in the MEL200 group (p<0.005), but overall survival (OS) was 67 months for MEL100 and 75 months for MEL200 (p NS). After MEL100, the duration of hospitalisation (p=0.05), of severe neutropenia (p<0.001) and of thrombocytopenia (p<0.001), transfusion requirements (p<0.002), incidence of mucositis (p=0.016) and fever of unknown origin (p=0.008) were all significantly reduced. MEL100 conditioning regimen was significantly less toxic compared with MEL200. MEL100 was similar to MEL200 in terms of CR rate and OS, while EFS was significantly longer in patients receiving high-dose Melphalan. MEL100 should represent the first alternative in elderly patients or in those with poor clinical conditions while MEL200 should be considered the standard care for younger myeloma patients in good clinical conditions.

Flow-cytometric monitoring of minimal residual disease (MRD) clearly identifies patients with high relapse risk in childhood acute lymphoblastic leukemia (ALL). A few studies have been reported so far on the clinical impact of immunophenotypic detection of MRD in adult ALL. We have recently shown that the detection of cyCD3+/TdT+ residual clones is a reliable prognostic factor in adult T-ALL treated with the Italian cooperative GIMEMA LAL 0496 protocol. To assess whether immunophenotypic detection of MRD can be effective in predicting the relapse in adult B-lineage ALL, as suggested by some Authors, we have studied the incidence of thir-

CO003

FLOW-CYTOMETRIC MONITORING OF MINIMAL RESIDUAL DISEASE IN ADULT B-LINEAGE ALL: ASSESSMENT OF THE FREQUENCY AND RELIABILITY OF LEUKEMIA-ASSOCIATED MARKER COMBINATIONS

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Flow-cytometric monitoring of minimal residual disease (MRD) clearly identifies patients with high relapse risk in childhood acute lymphoblastic leukemia (ALL). A few studies have been reported so far on the clinical impact of immunophenotypic detection of MRD in adult ALL. We have recently shown that the detection of cyCD3+/TdT+ residual clones is a reliable prognostic factor in adult T-ALL treated with the Italian cooperative GIMEMA LAL 0496 protocol. To assess whether immunophenotypic detection of MRD can be effective in predicting the relapse in adult B-lineage ALL, as suggested by some Authors, we have studied the incidence of thir-

CO004

LONG-TERM FOLLOW-UP OF INDOLENT LYMPHOMA PATIENTS TREATED WITH HIGH-DOSE CHEMOTHERAPY AND AUTOGRFTING: DURABLE CLINICAL AND MOLECULAR REMISIONS CAN BE ACHIEVED ONLY IN FOLLICULAR SUBTYPES

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The role of molecular monitoring of minimal residual disease (MRD) has been evaluate in 70 pts with advanced stage indolent lymphoma (NHL) [40 follicu-
lar (FCL), 11 of them with signs of histologic transformation, 14 small lymphocytic (SLL) and 16 mantle cell lymphomas (MCL). All received an intensified high-dose sequential (i-HDS) chemotherapy program including a final autografting phase: 61 pts were treated at disease onset, and 9 at relapse. The i-HDS program started in 1989 and consisted of 2 APO courses, 1 or 2 DHAP, etoposide 2 g/m², methotrexate 8 g/m², cyclophosphamide 7 g/m² and finally mitoxantrone 60 mg/m² plus melphalan 180 mg/m² followed by autografting. MRD was evaluated on peripheral blood progenitor cells (PBPC), bone marrow (BM) cell harvests, and in BM samples after autografting. It was assessed by PCR, using bcl-1, bcl-2 or IgH gene as tumor cell markers. In 60 of 70 patients (86%) a molecular marker was available (7 based on bcl-1, 30 on bcl-2 translocations, and 23 on IgH genes). A total of 160 PBPC and 46 BM harvests were analyzed before autografting. In 22 of 60 patients lymphoma cells were not detected in one or more PBPC and/or BM harvests: 19 of 35 (54%) FCL patients and only 3 (12%) out of 25 patients with non-follicular subtypes (p<0.025). The 12-year overall survival projections, according to FCL or non-FCL subtypes, are 76% and 49% respectively (p<0.05), while event-free survival curves are projected to 57% and 17% respectively (p<0.01). The collection of PCR-negative harvests and the achievement of post-transplant molecular remission (MR) were common among follicular subtypes (54% and 70% respectively), while they were not frequent among MCL and SLL (12.5% and 25%). Interestingly, 6 of 9 patients (66%) with transformed FCL could harvest PCR-negative cells. With a median molecular follow-up of 75 months, a 88% incidence of relapse was observed among patients never achieving MR, 61% in patients with mixed-PCR results, and 8% in those who maintained a continuous PCR negativity (p<0.001). The only relapsed patient with persistent PCR-negative BM samples was a MCL experiencing a localized central nervous system relapse. In conclusion, our findings indicate that: i) FCL or non-FCL subtypes show a significantly different behavior in terms of MR achievement; ii) durable PCR-negativity is strongly associated with a prolonged disease-free survival, on the contrary PCR-positivity is associated to a high relapse risk.

The Italian Cooperative Study Group on CML (ICSG on CML) has conducted a phase II study of imatinib in Ph+ CML patients in chronic phase who failed interferon (IFN). The primary endpoint of the study was the rate of major and complete cytogenetic response (CgR). Among secondary endpoints, the duration of the complete hematologic response (CHR) and of the major and complete CgR, overall survival, safety and compliance. Moreover, the kinetic of the molecular response was evaluated. From August to December 2000, 49 centers of the ICSG on CML enrolled 200 patients, of whom 191 were evaluable: 54% males, median age 48 yrs, median time from diagnosis to accrual 38 months. The 191 patients were enrolled according to 3 different categories of IFN failure: 32 (17%) for hematologic failure, 103 (54%) for cytogenetic failure and 56 (29%) for intolerance to IFN. The scheduled dose of imatinib was 400 mg daily, to be escalated (maximum 800 mg daily) in case of further refractoriness. This analysis has been conducted when the median time of observation was 26 months (range 12-36). Overall, 171 patients (89%) obtained a CHR. One hundred-eight patients obtained a major CgR during the first year of treatment: in 85/108 (or 44% of the whole patient population) the CgR was complete (CCgR). The CCgR was recorded only once in 9 cases: it was unstable and quickly lost. Of the remaining 76 cases of CCgR, 1 committed suicide and 8 lost the CCgR during the second year of observation. After 2 years of observation, 67 of the 85 complete responders (or 35% of the whole patient population) were still in continuous CCgR. Safety: the frequency of all adverse events (AEs), especially hematologic AEs, was maximum during the first months of treatment and decreased rapidly with time; in particular, the incidence of grade III/IV hematologic AEs, expressed as number of AEs per patient at risk per month, declined from 0.20 during the first quarter to 0.10 during the second quarter to 0.03 during the second six months of the 12-months trial time. Similarly,

CO005 IMATINIB IN PH CHRONIC MYELOGENOUS LEUKEMIA PATIENTS IN CHRONIC PHASE WHO FAILED INTERFERON: RESULTS OF A II PHASE STUDY


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the incidence of non-hematologic AEs of grade II and III declined from 0.15 and 0.03 during the first quarter to 0.06 and 0.01 during the second quarter and to 0.03 and 0.006 during the second six months of the trial time, respectively. Only four episodes of grade IV non-hematologic toxicity were recorded. Data on molecular response will be presented.

**CO006**
REDUCING TRANSPLANT RELATED MORTALITY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Transplant related mortality (TRM) is a major draw back for allogeneic hematopoietic stem cell transplantation (HSCT). In the present study we analyze the reduction of TRM in our Unit in 4 time periods: <=1985, 86-1990, 91-95, 96-2000. We included a total of 1033 consecutive allogeneic HSCT for hematologic malignancies, 865 with leukemia and 278 with a diagnosis other than leukemia; the latter included lymphoma, myeloma, myelofibrosis, myelodysplasia. Minimum follow up for survivors was 2.5 years. There were several major modifications during the four eras: increasing patient age (from a median age of 23 to 38 years), increasing use of alternative donors (from 5% to 42%), and less patients receiving total body irradiation (TBI) (from 97% to 52%). Also graft versus host disease (GVHD) prophylaxis has changed and is now almost exclusively combined cyclosporin and methotrexate. Acute GVHD grade III-IV significantly decreased (from 22% to 12% p=0.001), whereas overall chronic GVHD increased (from 16% to 36%, p=0.002). TRM has declined significantly from 46% to 25% overall (p<0.0001); major reduction was seen in acute GVHD as a cause of death (from 17% to 7%) and hepatitis/VOD (from 7% to 1.4%), whereas death due to chronic GVHD has remained stable at 3%. In the period 1996-2000 TRM for HLA identical siblings is 17%, for family mismatched donors it is 52% and for unrelated donors 40%. Relapse has remained stable at 30% (p=0.4) and survival has improved from 32% to 53% (p<0.0001). For first remission /first chronic phase patients receiving a graft from a matched siblings the current TRM is 16%, the relapse rate 34% and survival 72%. For unrelated donor transplants these figures are 36%, 11%, 60%. This study confirms the significant reduction of TRM despite increasing age, and increasing donor/recipient disparities. Relapse has remained unchanged, and consequently survival is improved. These figures should be considered when counseling patients for an allogeneic HSCT.

**CO007**
INCIDENCE AND PROGNOSTIC SIGNIFICANCE OF KARYOTYPE ABNORMALITIES IN DE NOVO PRIMARY MYELODYSPLASTIC SYNDROMES: A STUDY ON 352 PATIENTS
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In de novo MDS karyotype defects have an incidence of 33-80% and predominantly consist in terminal and in interstitial deletions. Except for the 5q-, cytogenetic abnormalities are not specific of any MDS FAB subtype or IPSS category, however they recognize peculiar biological and clinical entities and are well-defined prognostic indicators. The aim of the present study was to establish the incidence of chromosome defects in a large series of MDS patients, to correlate them with FAB subtype, to evaluate the impact of IPSS cytogenetic subgroups and of single chromosome aberrations with a still ill-defined prognosis on AML evolution and overall survival (OS). Conventional cytogenetics was performed on bone marrow cells at diagnosis and during the follow-up, using a trypsin-Giemsa banding technique. M etaphase cells were obtained from short-term unstimulated cultures and chromosome abnormalities were defined according to ISCN. In the period January 1990-December 2001 352 patients were cytogenetically studied at our Institution. Sixteen patients were classified as refractory anemia with ringed sideroblasts (RARS), 151 as RA, 111 as RA with excess of blasts (RAEB), 50 as RAEB in transformation (RAEB-t) and 24 as chronic myelomonocytic leukemia (CMML). A chromosome abnormality was discovered in 221 patients (62%), 10 classified as RARS, 66 as RA, 91 as RAEB, 40 as RAEB-t and 14 as CMML. As far as single abnormalities are concerned, a del(5)(q13-14q33) and a deletion of the long arms of number 20 (20q-) were found in 20 and in 9 patients, respectively classified as RA. Del(5)(q23q33), trisomy 8 (+8), monosomy 7 (-7), del(12)(p12) and del(7)(q31q34) were discovered in 10, 17, 8, 10 and 17 patients respectively and were seen in all MDS FAB subgroups. Complex defects were present in 32 patients, mainly advanced MDS. Considering IPSS, a chromosome pattern associated with a favourable, intermediate and poor clinical outcome was seen in 170, 121 and 61 patients. Kaplan Meier estimates of AML evolution and OS since diagnosis in relation to IPSS cytogenetic subgroup and to single defects were computed. Event rates per 100 person-year were calculated together with their 95%
implement. More importantly, the CD20 transgene did not affect the functionality of T lymphocytes with respect to allogeneic recognition and cytotoxic response, anti-EBV cytotoxic response, antigenic response to tetanus toxoid antigen, IL-2, IL-4, IFN-γ production, chemotaxis in presence of SDF-1, phenotype for several activation markers including HLA-DR, CD25, CD69, CD95, and T-cell repertoire. Finally memory cells, which would be functionally important in vivo, were efficiently transduced.

CO008
CHARACTERIZATION OF CD20 TRANSDUCED T LYMPHOCYTES AS AN ALTERNATIVE SUICIDE GENE THERAPY APPROACH FOR THE TREATMENT OF THE GRAFT VS. HOST DISEASE
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We have previously proposed the CD20 molecule as a novel suicide gene for T lymphocytes in the context of allogeneic bone marrow transplantation, since CD20 can be used as both a selection marker and a killer gene following exposure to the anti-CD20 therapeutic antibody Rituximab. We now report on the preclinical studies using this novel system, where the best transduction protocol, reproducibility, yield, feasibility and functionality of the transduced T lymphocytes have been investigated on a large donor series. Wild type human CD20 cDNA was transduced into human T lymphocytes using a Moloney-derived retroviral vector. Alternative protocols were tested employing either one or four spinoculations and stimulating T cells with PHA or CD3/CD28. One spinoculation alone was sufficient to obtain approximately 30% of CD20 positive cells within four experimental days. Four spinoculations significantly increased transduction to 60%. A small difference in transduction efficiency was observed between the two stimulation methods, PHA being marginally superior to CD3/CD28. Transduced cells could be purified on immunomass columns, with purity reaching 98% and yield being on average 50%. Finally, 86-97% of immunoselected T lymphocytes could be killed in vitro by Rituximab and complement. More importantly, the CD20 transgene did not alter the functionality of T lymphocytes with respect to allogeneic recognition and cytotoxic response, anti-EBV cytotoxic response, antigenic response to tetanus toxoid antigen, IL-2, IL-4, IFN-γ production, chemotaxis in presence of SDF-1, phenotype for several activation markers including HLA-DR, CD25, CD69, CD95, and T-cell repertoire. Finally memory cells, which would be functionally important in vivo, were efficiently transduced.

CO009
REGRESSION OF CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA AFTER ERADICATION OF HELICOBACTER PYLORI
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In the last years, Helicobacter pylori (HP) infection has been associated with some autoimmune diseases, especially the idiopathic thrombocytopenic purpura (ITP) and there is now an increasing evidence that the bacterium eradication can induce platelet recovery, at least in a part of chronic ITP patients. From 2000 to now, we have investigated 47 adult patients with chronic ITP (20 males and 27 females; median age 51 years, range 18-89). ITP was defined by idiopathic thrombocytopenia (platelets less than 100x10^9/L) when other causes has been excluded and bone marrow megakaryocytic hyperplasia. Methods: assessment of HP infection by urea breath test (UBT) and, whenever possible, by histological examination of specimens obtained by gastroendoscopy; not serum antibodies tests for active infection were used. Eradication: by a proton-pump inhibitor and two antibiotics for 1 week. Control of HP eradication: by UBT, two months after treatment. Platelet count monitoring: every 2 weeks for 1 month, then monthly and assessment at 6, 12 and 24 months. A study, by PCR of IgH gene, Igkappa and Iglambda genes, of the gastric B-cell population clonality, was also performed. Results: we have found 24 infected ITP patients (53.5%), whose 22 have been eradicated (91%) and 13 showed a marked increase of platelet count (more than 120x10^9/L) (60%). The median follow-up was 28.5 months (range 6-46); only 1 patient relapsed 7 months after eradication. Responders by intention to treat were 54%. B-cell population of gastric tissues has been found clonal in 50% of 10 examined cases. A review of the literature, shows that 328 ITP cases has been studied up to now, of which 68.2% resulted HP infected, 73.1% eradicated and 63.7% responsive to eradication treatment. The responders by intention to treat were 41.5%. Discussion. In our ITP patients, the prevalence of HP infection appears to be higher than in Italian general healthy population, but
not if compared with age distribution. The bacterium eradication is highly effective in a remarkable percentage of patients. The study of gastric B-cell clonality might shed light on a possible role in the pathogenesis of ITP with HP infection. Conclusions. Search of infection and HP eradication treatment seem to be recommendable at diagnosis of ITP to avoid immunosuppression, splenectomy and their discomfort and side-effects, in a consistent part of patients.

C001
A RANDOMIZED CLINICAL TRIAL OF ORAL ANTICOAGULANT THERAPY IN PATIENTS WITH THE ANTIPHOSPHOLIPID SYNDROME: THE WAPS STUDY

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The WAPS study (Warfarin in the AntiPhospholipid Syndrome) is a multicenter randomized clinical trial with two objectives: to compare high-dose warfarin (PT INR 3.0-4.0) vs. conventional treatment (warfarin with PT INR 2.0-3.0 for venous and aspirin for arterial thrombosis) in patients with the antiphospholipid syndrome; b) to evaluate the clinical outcome of non-randomized patients in a parallel observational arm of the study. 462 consecutive patients (M/F 127/335; median age 39 years, range 15-83) with lupus anticoagulant or moderate to high titers of anticardiolipin antibodies have been enrolled. Of these, 109 (24%) were eligible for randomization, whereas 353 (76%) were included in the observational group because of: asintomaticity (146, 41%), excessive bleeding risk (25, 7%), absolute need for high-dose warfarin (57, 16%), patient’s unwillingness to participate (97, 27%) or other exclusion criteria (28, 8%). Median follow-up in both groups was 36 months (range 0-63). Actual PT INR of the 109 randomized patients was assessed at 3, 6, 12, 24 and 36 months. Median PT INR values of patients randomized in the high-dose group (n=54) was 3.1, 3.2, 3.1, 3.3 and 3.3, whereas in the conventional group (n=55) was 2.3, 2.5, 2.6, 2.5 and 2.5, respectively. An analysis of the main end points of the study was carried out in the total population, divided into randomized (n=109) and observational (n=353) patients. All cause mortality (4.6% vs. 3.4%) and thrombotic events (8.3% vs. 11.3%) were similar between the two groups. However, both major (4.6% vs. 0.8%, p=0.0206) and total bleeding (22% vs. 7.4%, p<0.0001) were significantly more frequent in the randomized patients. The final analysis of results in the two randomized arms of the study is currently ongoing and will be presented.

C001
VALPROIC ACID PLUS RETINOIC ACID INDUCE MYELOID DIFFERENTIATION IN CHEMOTHERAPY-RESISTANT ACUTE MYELOID LEUKEMIA PATIENTS


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The anti-epileptic drug valproic acid (VPA) has been recently shown to possess inhibitory activity against histone deacetylases. In vitro treatment of fresh acute myeloid leukemia (AML) blasts showed that, in combination with retinoic acid (RA), VPA triggers myeloid differentiation. Pre-clinical studies in murine models of leukemia, renal and lung metastases showed that VPA has anti-tumoral activity through a differentiation mechanism. We designed a phase II clinical study in which VPA was combined with RA (VPA-RA) in the treatment of non-APL (AML). Four chemotherapy-resistant AML patients (age of 54, 61, 31 and 54 years), not eligible for additional intensive therapy, were treated at the Hematology Unit of the University La Sapienza of Rome between December 2000 to October 2002. VPA (Depakin® Sanofi-Wintrop) was administrated from day 1 to day 28, at the initial dosage of 10 mg/kg/die p.o. with dose escalation until optimal VPA plasma levels (80-110 µg/ml). RA (Vesanoid® Roche) at the dosage of 45 mg/m² p.o./d, divided in two administrations, was added at optimal VPA plasma levels or at day 14 to day 28. Peripheral blood and/or bone marrow samples were collected at day 0, 3, 7, 14, 21, 28 for the evaluation of histone acetylation and for morphologic, immunophenotypic, cytogenetic, and molecular studies. Two patients had a history of MDS, while the remaining two had FAB M0 and FAB M2 de novo AMLs, respectively. Of the four patients, one had normal karyotype, one a pseudodiploid (der(12)), one a hyperdiploid (+8) K, and one a complex K with a 7q- alteration. Pre-treatment leukemic infiltration ranged from 61% to 95%. VPA plasma level >80 µg/ml was reached after 9, 11, 21, 23 days, respectively. In three patients VPA-RA treatment induced hyperleukocytosis (>50x10⁹/L) at day 16, 21 and 24, respectively, treated with chemotherapy (HU in two cases and low dose Ara-C in 1 case). Hematological improvement (>50% decrease in packed red blood cell or platelet transfusion requirement) was observed in one case while a stable disease and a disease progression were observed in two and one cases, respectively. All patients showed features of myeloid-mono-
cytic differentiation of the leukemic clone, as revealed by morphologic, cytochemical, and immunophenotypic analyses and Q-RT-PCR of myeloid gene expression (GATA 1, MPO, CSF2Rβ, etc.). High degree of myeloid differentiation correlated with early achievement of therapeutic VPA plasma levels and histone hyperacetylation, as measured by immunocytochemistry and immunoblotting using antiacetylated histone H3 antibodies. Finally, differentiation of the leukemic clone was proven by FISH analysis showing the presence of the +8 and 7q- in maturing elements in patients whose leukemia blasts carried these cytogenetic lesions. VPA-RA combinatorial is a well tolerated treatment that induce phenotypic changes of myeloid-monocytic differentiation of the leukemic clone through chromatine remodelling. Further studies are needed to optimise this regimen with the aim of improving clinical response in leukemia patients.

CO012
WT1 IS A MOLECULAR MARKER OF HYPEREOSINOPHILIC SYNDROME (HES) USEFUL TO PREDICT RESPONSE TO IMATINIB THERAPY
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The hypereosinophilia is a characteristic feature of clonal hematopoietic disease defined as HES in which the eosinophils have been demonstrated to be part of the malignant clone. An increasing number of HES patients have been demonstrated to be responsive to low doses of Imatinib, and the rational of the response is, at least in some cases, the presence of a molecular translocation recently described (FIP1L1-PDGFRα). The aim of this study is to identify a molecular marker able to distinguish between the hypereosinophilia associated with HES from the cases of reactive hypereosinophilia associated for example with allergic pathologies, parasite infections or vasculitis. Moreover, we validated an in vitro assay able to predict the response to Imatinib therapy in the cases identify as HES. The Wilms tumor gene (WT1), has been demonstrated to be overexpressed in a number of hematologic malignancies including AML, ALL, MDS or CML and it represents a molecular marker useful for detection of the presence of leukemic cells. Using a Real-Time quantitative PCR (TagMan) assay we analyzed WT1 expression levels in 7 cases of HES treated with Imatinib (3 of them were respondent and 4 resistant) and 5 cases of reactive hypereosinophilia. As control group we analyzed 52 PB and 15 BM from healthy volunteers. We found that the majority of normal PB samples scored negative for WT1 expression and the remaining cases expressed very low levels with a median value of 5 WT1 copies/10^4 ABL copies (range 1-22). Normal BM expressed a median value of 78 WT1 copies/10^4 ABL copies (range 3-180). In all the HES cases WT1 expression was increased above the normal range, in both BM and PB with a median value of WT1 copies/10^4 ABL of 254 and 56 respectively. By contrast, in all the cases of secondary eosinophilia WT1 was found within the normal range. In addition we test the possibility to predict the response to Imatinib therapy using an in vitro assay that has been previously tested for CML patients. We incubated the BM cells of HES patients with or w/o 10 µM Imatinib for 18 hours and we evaluated WT1 expression after incubation. In CML patients the WT1 was significantly decreased in incubated BM cells from cytogenetically responding patients but not in those from resistant ones. Accordingly, in the three patients who revealed responsive to Imatinib therapy, WT1 was significantly inhibited by the in vitro incubation. By contrast, no downmodulation of expression was detected in the 4 cases of unresponsive patients. These data allow to identify a molecular marker of HES and to validate an in vitro assay which, as in CML patients, allows to identify the patients responsive to Imatinib independently on the underlying molecular mechanism.
LYMPHOPROLIFERATIVE SYNDROMES

CO013
SPLENIC MARGINAL ZONE LYMPHOMA WITH AND WITHOUT CIRCULATING VILLOUS LYMPHOCYTES. A RETROSPECTIVE MULTICENTER STUDY ON 99 CASES

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Introduction. SMZL VL+/VL− is a recently recognized clinicopathologic entity but only a few series have been published so far. It has been acknowledged that in the bone marrow the disease frequently shows an intrasinusoidal infiltration pattern. However, issues such as the minimal diagnostic data set, the prognostic factors and therapy are still not completely clarified. Here we present a multicenter retrospective analysis on a series of 99 patients. We considered four diagnostic hallmarks in patients with splenomegaly and without clinically significant peripheral lymphoadenomegaly: (i) presence of light chain restricted CD19/CD20 positive circulating lymphocytes, negative for CD5/CD10/CD23/CD25; (ii) villous lymphocytes > 10% in the peripheral blood; (iii) Intrasinusoidal bone marrow infiltration pattern and (iv) Diagnosis of SMZL on surgically removed spleen. The diagnosis was considered definite when an unambiguous spleen histology and/or a clone showing the typical morphology and immunophenotype was present. Histologic data evaluation was done on the original pathological reports. Results. The disease occurs mostly in elderly males (median age 63.5 years M/F ratio 1.7). Splenomegaly was the commonest presenting feature. It was felt more than 4 cm below the left costal margin in 66% of cases and hepatomegaly was recorded in 24%. Anaemia was found in 57 patients (median 11.6 g/dl; range 6.8-17). Thrombocytopenia and/or leukopenia were present in about one third of cases but were severe in only one (platelets < 20.000 mm−3 - neutrophils < 500 mm−3). 41 patients had lymphocytosis (median 4126 mm−3). The bone marrow was infiltrated in all cases. The intrasinusoidal infiltration pattern was detected alone or along with a nodular or interstitial component in 62% of cases. A Monoclonal Component was found in 27% of cases and two thirds were of IgM type. Twenty percent of the patients were HCV positive. Villous lymphocytes were found in 45% of cases. Accordingly, 54 patients were diagnosed as SMZL(vl+) and 45 as SMZL (vl−). None of the above indicated presenting features differed between the two groups, but leukocytosis > 30.000, lymphocytosis > 5.000 and neutropenia < 1000 were significantly more frequent in the latter. Thirty patients were placed on watch and see policy until disease progression; 79% of them are projected to be alive at 84 months since diagnosis. The remaining patients received one or more lines of treatment (1-4). Overall, thirty patients underwent splenectomy (sixteen at diagnosis) with a sustained response lasting from 6 months to 7 years (median 5 years) in most of them. In all cases the histologic picture of SMZL was found, confirming the reliability of the diagnosis already formulated with a non invasive approach. The outcome of the patients primarily treated with chemotherapy or splenectomy did not differ significantly, (OS at 5 yrs: 68% vs 60%). TTF and OS did not differ between SMZLvl+ SMZLvl−. For the whole series median survival has not been reached, and 75% of patients are projected to be alive at 7 years. In a multivariate analysis anemia (Hb < 12 g/dL) was a significant adverse prognostic factor for both TTF and OS, while thrombocytopenia, lymphopenia and splenomegaly > 4 cm were associated with a worse TTF. Conclusions. SMZL is a indolent disease with up to one third of cases not requiring therapy at diagnosis. The presence of villous lymphocytes does not influence the prognosis. The bone marrow intrasinusoidal infiltration pattern is a valuable hallmark for the diagnosis. Anemia at diagnosis identifies a group with a significantly worse TTF and OS.

CO014
PROGNOSTIC SIGNIFICANCE OF ANGIOGENIN SERUM CONCENTRATIONS IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Human angionenin is a potent inducer of angiogenesis. The association between angionenin and cancer progression and poor outcome in solid tumors has been
documented, but its significance in leukemias has not been evaluated. We measured, using an ELISA technique (Quantikine Human Angiogenin Immunoassay, R & D Systems), serum angiogenin levels in 78 previously untreated Binet stage A B-cell chronic lymphocytic leukemia (CLL) patients. Although no difference could be found with age- and sex-matched healthy controls, increased angiogenin serum level was associated with higher LDH (p=0.03) and β2-m (p=0.007) concentrations. Angiogenin did not reflect the extent of bone marrow (BM) angiogenesis as evaluated by microvessel number in 32 patients (r=-0.106; p=0.611). The same applied when correlation were attempted with circulating levels of vascular endothelial growth factor (VEGF) (p=0.873) and basic fibroblastic growth factor (FGF-2) (p=0.421). As genomic aberrations are independent predictors of disease-progression, their association with angiogenin was sought. When the 24 patients with available data were stratified into the four major cytogenetic categories (normal karyotype, 13q as a sole aberration, 12q trisomy, 11q or 17p deletion) and aberrations were compared with angiogenin serum levels, no correlation was found (p=0.651; Kruskall-Wallis). A cut-off of angiogenin serum levels corresponding to median (i.e., 330 ng/mL) or higher identified later upstaging and longer progression-free survival (PFS) (p=0.03). Although in multivariate analysis only Rai substages (p=0.00001), peripheral blood lymphocytosis (p=0.009) and serum levels of FGF-2 (p=0.001) retained their prognostic significance, angiogenin could be incorporated into the Rai substages thus leading to the identification of the following risk categories: stage 0 (angiogenin > 330 ng/mL); stage 0 (angiogenin < 330 ng/mL) + stage I-II (angiogenin < 330 ng/mL); stage I-II (angiogenin < 330 ng/mL). The 40-month PFS was as follows: 85%, 65%, 25% (χ2 for trend=6.33; d.f.=1; p=0.01). In conclusion, serum levels of angiogenin predict clinical outcome and helps to refine the prognosis of early CLL patients.

We report the characterization of a 28 kDa protein sharing an antigenic determinant with the intracytoplasmic portion of the human IRTA1 protein, as revealed by a newly generated mAb (B-28b). Expression of the 28 kDa molecule in normal lympho-hemopoietic tissues was different from that expected for IRTA1 (marginal zone B-cell subset), being selectively detected in the cytoplasm of all mature B cells, with highest levels in plasma cells. Analysis of the 28 kDa protein by 2D-PAGE and highly sensitive electrospray ionisation tandem mass spectrometry yielded several peptide spectra that corresponded to human protein TPD52, whose expression had been previously described only in epithelial tissues. Specific B-28b reactivity with TPD52 was confirmed by immunostaining and Western blotting of TPD52-transfected cells. This study first identifies the epithelium-associated TPD52 protein as a new B-cell/plasma cell-associated molecule whose expression pattern differs from that of all the other B-cell/plasma cell-associated antigens. Notably, TPD52 represents the first example of a pan-B-cell-associated protein whose levels are increased during differentiation to plasma cells. Moreover, our findings suggest that, due to its reactivity with a fixative-resistant epitope of TPD52, B-28b may serve as valuable tool for the routine diagnosis of B-cell lymphomas/myeloma and for the differential diagnosis between ALCL and Hodgkin’s disease.

**CO015**

THE HUMAN TUMOR PROTEIN S2 (TPD52): A NOVEL B CELL/PLASMA CELL ASSOCIATED MOLECULE WITH UNIQUE EXPRESSION PATTERN

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IRTA1 is a novel surface B-cell receptor related to Fc-receptors, IRS and CAM family members and we mapped for the first time its distribution in human lymphoid tissues, using newly generated specific antibod-
It has single-agent activity in low-grade and diffuse B-cell lymphomas and acute leukemias, new combination strategies that have been successful in curable malignancies such as diffuse large-cell non-Hodgkin’s lymphoma (NHL). In CLL, the single-agent activity of rituximab, used at standard NHL doses, has been marginal. However, on the basis both of the recent in vivo demonstration that fludarabine has a synergic cytotoxic activity with rituximab and the safety and efficacy of rituximab used in combination with chemotherapy in NHL, we conducted a study in our Institution in order: i) to determine the ideal therapeutic schedule of rituximab when combined sequentially with fludarabine in B-CLL, and ii) to evaluate the clinical response and outcome of this group of pts as compared with a historical subset of 35 pts treated with fludarabine alone. Between October 1998 and November 2002, twenty-nine CD20+ B-CLL untreated pts, median age 60 years, received fludarabine (25 mg/m²) intravenously daily on days 1 through 5, with the treatment repeated every 28 days for a total of 6 cycles. Pts with stable disease or better (complete and partial remissions defined by NCI criteria) were then treated with 4 weekly doses of rituximab (375 mg/m²). The median time from treatment with fludarabine was 65 days (range 15-135). Only 5/29 pts experienced mild infusion-related symptoms consisting of fever, chills and rigors, during the first infusion of rituximab and no patient presented severe (III or IV grade according to the WHO) infective toxicity. Based on the NCI criteria, 27/29 (93%) pts achieved a complete remission (CR), 1/29 (3%) partial remission (PR) and 1/29 (3%) no response (NR). The median duration of CR and PR was 15 months (range 10-38). Moreover, B-CLL pts in CR after combined therapy showed a significantly higher CD20 mean fluorescence intensity, evaluated by flow cytometry, than did pts in PR or with no response or progressive disease (46.4 vs 22.6; p=0.007). Therefore, we performed a historical comparison with 35 pts, median age 61 years, treated with fludarabine alone as front-line therapy, between January 1996 and October 1998. Within this subset, 13/35 (37%) pts achieved CR, 11/35 (31%) PR and 11/35 (31%) NR or progression. Noteworthy, B-CLL pts treated sequentially with fludarabine and rituximab had a significantly higher overall response rate (CR+PR) (96% vs 68%; p=0.00002), a longer PFS from treatment (83% vs 32% at 2 years; p=0.001) and a longer overall survival (OS) (95% vs 66% at 8 years; p=0.01). Therefore, the sequential combination of fludarabine with rituximab was safe and significantly increased CR rate, PFS and OS as compared with fludarabine. In conclusion, we can say that MoAbs, such as rituximab, in combination with chemotherapy, may exert an important role in eradicating minimal residual disease in B-CLL, allowing a better outcome.
ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING FOR POOR RISK CHRONIC LYMPHOCYTIC LEUKEMIA. PRELIMINARY RESULTS OF A PILOT STUDY

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Chronic lymphocytic leukemia (CLL) is a slowly progressing lymphoproliferative disorder but a subset of patients show phenotypic and molecular features predicting a more aggressive course at the onset of the disease or after an indolent phase. Autologous bone marrow transplantation could provide longer remission duration than standard therapies but does not seem to be a curative approach for this disease. Allogeneic stem cell transplantation might be an option for poor risk CLL since the disease is known to be highly sensitive to graft-versus-leukemia (GVL) effects but has been associated with an extremely highly transplant-related mortality, particularly in elderly patients. To avoid toxicity while fully exploiting the GVL effect associated with this disease we explored, in a pilot study, a reduced-intensity regimen (RIC) for very high risk CLL patients. In particular 12 patients [median age 55 (46-60) years] with advanced Binet stage CLL (7 B and 5 C) and high risk cytogenetic abnormalities underwent nonmyeloablative therapy. The conditioning regimen consisted of two cycles of Fludarabine 30 mg/m²/day for 3 days and therapy. The conditioning regimen consisted of two cycles of Fludarabine 30 mg/m²/day for 3 days and Cyclophosphamide 300 mg/m²/day for 3 days (FC) every 28 days. Immunosuppression included 200cGy TBI (day 0) and treatment with cyclosporine and mycophenolate mofetil acid. Donor leukocyte infusions were given after transplant for persistent malignancy and/or mixed chimerism. The treatment was well tolerated with very low myeloablation and day 100 transplant related mortality (TRM) = 0. Complete remission was achieved in 8 of 11 valuable patients, and 3 patients achieved a very good partial remission. At 12 months, acute grade I-III GVHD occurred in 16% of patients and chronic GVHD occurred in 25%. One-year event free survival was 91%. At a median follow-up of 19 months, 11/12 patients achieved completed chimerism. Overall survival was 75%, 1 patient died of progressive disease, 1 patient died of GVHD and 1 of toxic hepatitis. Cytomegalovirus reactivation was seen in only 2 patients. Our data show extremely encouraging results in terms of CR rate and TRM in this group of patients with an otherwise dismal poor prognosis.

ACUTE MYELOID LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES

ITALIAN REGISTRY OF MYELODYSPLASTIC SYNDROMES: PRELIMINARY EPIDEMIOLOGICAL DATA

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The interest in biological and clinical studies on myelodysplastic syndromes (MDS) is greatly increased in this last decade. However, the incidence and the epidemiological features of these hematological disorders are still very poorly defined. All the epidemiological data up to now available, provided by various regional cancer registries, have suggested that MDS are much more common than previously thought and at least as frequent as acute myeloid leukemia (AML). As MDS diagnosis and classification are difficult, the registration of patients in population-based registries is far from adequate and a registry devoted to MDS is absolutely mandatory for truly representative statistics. The Italian MDS registry has been developed on January 2000 at University of Pavia Medical School and is located at Collegio Ghislieri Foundation, Center for Research and Communication. The first aim of the Registry is the collection of adequate and complete epidemiological data from all over the Italian country. Even if the Registry has not yet determined the crude incidence rate and the age-specific incidence rate of MDS, it has collected some preliminary epidemiological data from 1024 MDS Italian patients. In our series 605 patients were males and 419 were females and therefore the sex ratio was 1.4. The mean age of our patient population was 75.4 years (range 16-98). Considering age, 11 patients (0.9%) were less than 30 years, 17 (1.6%) 30-40, 23 (2.2%) 40-50, 88 (8.5%) 50-60, 266 (25.9%) 60-70 and 427 (41.6%) 70-80; 192 patients (18.7%) were above 80 years. When 70 years was chosen as the age to subdivide patients in two groups, MDS patients having <70 years were 405 (39.5%), while those having >70 years were 619 (60.4%). Twenty-nine percent of patients were smokers and 18.6% had a familial history of cancer. A previous exposure to environmental carcinogens occurred in 16.2% of patients, while a previous exposure to chemotherapy performed for another cancer occurred in 6.4% of patients. As far as the MDS FAB subtype is concerned, 505 patients (49.3%) were diagnosed as RA, 90 (8.7%) as RARS, 272 (26.5%) as RAEB, 74 (7.2%) as RAEB-T and 83 (8.1%) as CrM L. Considering the 897 patients (87.5%) evaluable for the International Prognostic Scoring System, 319 (35.5%) were classified as low-risk MDS, 219 (24.4%) as intermediate-1 risk, 185 (20.6%) as
intermediate-2 risk and 174 (19.4%) as high-risk. Our study confirms that subjects aged more than 70 years are those mainly affected by MDS. Therefore, the aging process plays a pivotal role in the development of myelodysplasia, as may cause a compromise in stem cell reserve, or alternatively may favoured the progressive acquisition of genetic defects. These last may lead to an increase of apoptosis in patients with low-risk MDS, and to an excessive cell proliferation in advanced disease. Obviously this pathogenetic pathways, related to age, may be stressed by the action of mutagenic factors. In conclusion, the collection of precise and complete epidemiological data from defined geographical area (prospectively all over Europe) might shed light on MDS etiopathogenesis and be very useful to identify risk factors within the general population.

CO020
DOWN-MODULATION OF CEBPA TRANSCRIPTION FACTOR IN CORE BINDING FACTOR ACUTE MYELOID LEUKEMIAS
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The loss of transcriptional control is a common mechanism of leukemogenesis. The CCAAT enhancer binding protein α (CEBPα) is an important transcriptional regulatory element which plays a key role in the differentiation of the myeloid compartment. A significant percentage of AML patients are characterized by abnormalities in CEBPα function which can be disrupted with different mechanisms, in particular by point mutations of the gene. These latter have been identified in patients with normal karyotype. In promyelocytic leukemias the interaction between PML-RAR fusion protein and CEBPα leads to the inhibition of the DNA binding. Recent data reported the down-modulation of CEBPα in the subgroup of AML characterized by the translocation t(8,21) and this latter mechanism is probably responsible for the block of differentiation observed in this subset of AML. By contrast no inhibition of gene expression was reported at present in other subtypes of AML. The present study was aimed to assess whether the down-modulation of CEBPα expression could be a possible mechanism which leads to the arrest of the differentiation process in different subtypes of AML other than t(8,21). We analyzed the bone marrow samples from 130 AML patients (10 FAB M0, 17 FAB M1, 17 FAB M2, 18 FAB M2 with t(8,21), 22 FAB M3, 19 FAB M4, 16 FAB M4 with inv(16), 8 FAB M5, 3 FAB M6). Quantitative Real Time PCR was carried out using iCycler (Biorad) and the values obtained were expressed as CEBPα copy number/10⁴ ABL copies used as housekeeping gene. The AML cases with t(8,21) and inv(16) showed a significant lower amount of CEBPα transcript respect to all the other cases (p=0.0001 and p=0.0002 respectively). If we consider the cases of AML FAB M2 with t(8,21) and M4 with inv(16) respect to the same FAB subtypes with normal karyotypes, the difference is even more significant (p=4.7×10⁻⁶ for t(8,21) cases and 3.5×10⁻⁶ for inv(16) cases). No significant differences were detected among the other subgroups. In addition 18 AML patients were tested for CEBPα expression during follow-up. 7 out of 18 cases were characterized by the translocation t(8,21), 5 by inv(16) and the remaining cases showed a normal karyotype. In Core Binding Factor (CBF) AML patients who obtained the remission after chemotherapy, CEBPα expression was significantly up-regulated during follow-up and reached values similar to those detected in BM from healthy volunteers. Western Blot was performed in the samples taken at diagnosis to assess the amount of CEBPα protein. We found that the amount of CEBPα protein in the BM samples of CBF AMLs at diagnosis was lower respect to the amount detected in all the other subtypes. These data suggest that the down-modulation of expression is a possible mechanism of disruption of the CEBPα pathway shared by both types of AML characterized by the rearrangement of the CBF.

CO021
TREATMENT IMPLICATIONS FROM A TWO-STEP, INCREASING-INTENSITY REMISSION INDUCTION PHASE FOR STANDARD- AND HIGH-RISK ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA
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Background and aim of study: in adult AML, postremission consolidation techniques may vary considerably in relation to pretreatment risk class; however, despite sound clinical and prognostic evidence, the adoption of a risk-adapted remission induction strategy has been seldom if ever considered. With regard to the latter point, we review complete remission (CR) and failure rates following a two-step, increasing-intensity induction in adult patients with SR and HR AML. Patients and methods: in NIGL-AML trial 00-01, CR induction for unselected adults with AML aged up to 65 years consisted of course #1 (ICE-G: IDR 12 mg/m²/d dd 1-3, VP16 100 mg/m²×2/dd dd 1-5, ara-C 100 mg/m²×2/dd dd 1-7, G-CSF from d 8). ICE-G-refractory cases were immediately transferred to an intensified induction course #2 (HAL-G: ara-C 3 g/m²×2/dd dd 2,3 and 9,10; IDR 17.5
the expression of selected adhesion molecules in 270
well as immune altered surveillance. We have studied
molecules may interfere with the adhesive properties as
specific cell surface receptors. The expression of these
and leukemogenesis. These interactions are mediated by
endothelial cells are important in normal hematopoiesis
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Interactions between hematopoietic cells and
endothelial cells are important in normal hematopoiesis
and leukemogenesis. These interactions are mediated by
specific cell surface receptors. The expression of these
molecules may interfere with the adhesive properties as
well as immune altered surveillance. We have studied
the expression of selected adhesion molecules in 270 newly
diagnosed cases of AML. The blast cells were
analysed by flow cytometry; monoclonal antibodies used
were directed to β-1 integrins (CD49a, CD49b, CD49d,
CD49e, CD49f), β-2 integrins (CD11a, CD11b, CD11c,
CD18), ICAM-1, CD62L, CD31 and CD44. A given marker
was regarded as positive if expressed on more than 20%
of cells in the blast gate. In order to define the
clinical relevance of these molecules we have analysed
their correlation with FAB subtype, karyotype, response
to therapy and prognosis. One hundred and 46 patients
(pts) were males (54%) and 124 females (46%); medi-
an age was 55 years (range 18-86). FAB categories were
as follow: M0 30 (11%), M1 57 (21%), M2 57 (21%), M3
27 (10%), M4 37 (14%), M5 59 (22%) and M6 3 (1%).
In the patient population were also included 35 cases of
secondary AML (SAML) (13%).

In the patient population were also included 35 cases of
secondary AML (SAML) (13%). The pts were treated
according to the current EORTC/GIMEMA protocols. The
expression of β-1 integrins was significantly associated
with M4/M5 subgroups; in fact CD11a and CD18 were
expressed in 100% of M5 (p<0.001), CD11b in 99% of
M5 and the lowest expression was observed in M3 where
CD11c was not expressed at all (p<0.001). CD54 was
found in 91% of sAML (p<0.001), in 77% of M0 and in
82% M5 (p<0.001). CD49f was expressed mainly in M0
(72%) with lower expression in M4/M5 (p=0.03). CD62L,
CD31 and CD44 were expressed heterogeneously within
the FAB subgroups. CD11a expression was significantly
associated with high risk cytogenetics (p=0.02). AML-
M3 cases were excluded when the analysis of response
to treatment was performed. CD11b and CD11a were
associated significantly with a low complete remission
rate (p<0.001) and short duration of overall survival
(p=0.003), respectively. We conclude that the expres-
sion of adhesion molecules in AML may be relevant in
terms of classificative purposes and prognostic impact.

CO023
SELECTIVE INHIBITION OF MEK1 PHOSPHORYLATION ENHANCES
ARSENIC TRIOXIDE-MEDIATED APOPTOSIS IN ACUTE
PROMYELOCYTIC LEUKEMIA

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It is now common knowledge that blast cells in most
cases of acute myelogenous leukemia (AML) present high
activation levels of extra cellular signal-regulated kinases
1/2 (ERKs 1/2) and of kinases immediately upstream of
ERK, known as mitogen-activated protein (MAP)/ERK
kinases (MEKs). Furthermore, the studies conducted by
our, and other, work groups have clearly demonstrated
that downmodulation of the phosphorylation of MEK1 in vitro inhibits the proliferation and induces the apoptosis of AML primary blasts. Treatment of acute promyelocytic leukemia (APL) with Arsenic Trioxide (ATO), either alone or in association with other compounds such as Idarubicin, has proved to be clinically efficacious. The aim of this study was to investigate whether the association of ATO with MEK1 phosphorylation inhibitors has, in fact, the capacity in vitro to enhance ATO-induced apoptosis in the NB4 cell line and in APL primary blasts. To this end, we used the inhibitors of MEK1 phosphorylation PD98059 (Cell Signaling Technology, Beverly, MA, USA) and PD184352 (Pfizer Global Research & Development, Ann Arbor, MI, USA). Both in the NB4 cell line and in APL primary blasts, a 3-hour pre-incubation period of the cells with PD98059 or PD184352 induced a significant increase in ATO-mediated apoptosis with either an additive or synergic effect, depending on the dosages and times of exposure. When primary cells were treated using doses of 0.5-2 µM of ATO, which are within the range observed in the plasma of APL patients treated with ATO, the resulting data were particularly significant. Interestingly, the pre-incubation period with PD98059 or PD184352 was able to completely restore sensitivity to ATO, inducing apoptosis, with the NB4 cell line rendered ATO-resistant (NB4-AsR). From the molecular point of view, treatment with ATO alone was associated with a significant increase of the activity of the pro-survival molecules ERK1/2 both in NB4 and in NB4-AsR and in the primary blasts. Pre-incubation with PD98059 or PD184352 induced downmodulation of MEK1 phosphorylation and of ERK1/2 activity, de-phosphorylation (active form) of the Bad pro-apoptotic molecule, as well as a marked increase of the PARP fragment, an early sign of apoptosis. In conclusion, our results and the existence of formulations for oral use of PD184352 or its derivates in clinical trials, suggest that the association between inhibitors of MEK1 and ATO could be tested as a therapeutic tool in APL.

FLT3-ITD IN ACUTE PROMYELOCYTIC LEUKEMIA IS ASSOCIATED WITH SPECIFIC BIOLOGICAL CHARACTERISTICS AND WORSE PROGNOSIS

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Acute promyelocytic leukemia (APL) is characterized by the presence of a specific chromosomal translocation, i.e. t(15;17), that leads to the formation of a chimeric gene named PML/RARα. Recently, molecular defects in the FLT3-kinase gene have been reported to be frequent in APL patients, i.e. an internal tandem duplication (ITD) in exon 11 and point mutations in codon D835/I836 (TKD). These alterations lead to ligand-independent activation of the kinase activity and thus may play a key role in leukemogenesis, cooperating with PML/RARα as already shown in a mouse model. In the period 1993-2001, at our Institution 42 patients were diagnosed as affected by APL. WBC count was 500-234000/mcl (mean 31200, median 10600); PLT were 5000-98000 (mean 29800, median 18500); FAB classification was M 3 in 35 out of 42 (83.3%) and variant M 3 (M3v) in 7/42 (16.7%); PML/RARα breakpoint was BCR1 in 23/42 (54.8%), BCR2 in 2/42 (4.7%) and BCR3 in 17/42 (40.5%). In this consecutive series, we investigated the presence of FLT3-ITD by PCR both on genomic DNA and on cDNA with fluorescent primers and subsequent capillary electrophoresis on the ABI Prism GeneScan 310. ITD was recorded in 10/42 patients (23.8%); the mutated vs. wild-type (WT) ratio was calculated and in no patient was observed loss of WT allele, in one case a biallelic disease was present. The length of the duplicated fragment was 21-90 base pairs. ITD+ patients always showed WBC count more than 10000/mcL and significantly higher than ITD- patients: 17960-234000 (mean 83300, median 47 700) vs. 500-101000 (mean 14400, median 2100) respectively, p<0.0002. PLT values were not significantly different in the two groups of patients. There was a significant association between M 3v FAB phenotype and ITD+ (χ² p<0.03); moreover, ITD+ was more frequent in patients with PML/RARα BCR3 breakpoint (χ² p<0.03). The TKD point mutation was studied by digestion of PCR amplions with EcoRV and eventual direct sequencing; the mutation was present in 3/42 (7.1%), one of these was TKD+ patients was ITD+ too. Two of these were D835Y, the third patient (also ITD+) showed a I836F mutation. All patients was ITD+ too. Two of these were D835Y, the third patient (also ITD+) showed a 1836F mutation. All 42 patients were treated according to GIMEMA AIDA protocol, with chemotherapy + ATRA; 33/42 (78.6%) patients obtained a CR after induction therapy. Nine patients died early (ED): 6/9 were ITD+, 7/9 died for hemorrhagic events. Five patients relapsed, one ITD+ (death in relapse at 790 days) and 4 ITD- (all alive, 3 in CR and one in 2nd relapse). Early death was associated with ITD+ (χ² p<0.003), probably due to the more frequent hyperleukocytosis in this group of patients. Of the 3 TKD+ patients, 2 are alive in CR and one (also ITD+) had an ED. Overall survival (9 year follow-up, median 3 years) is significantly different: 81.2% for ITD- vs. 30% for ITD+, p<0.0007. So, while TKD mutations are relatively rare and their prognostic significance is difficult to determine, ITD is associated with defined clinical and biological characteristics (higher WBC, FAB M 3v and BCR3 breakpoint) and worse prognosis.
ACUTE LYMPHOID LEUKEMIAS

**CO025**
MYLOTARG (GEMTUZUMAB OZOGAMICIN) HAS THERAPEUTIC ACTIVITY AGAINST CD33+ ACUTE LYMPHOBLASTIC LEUKEMIAS IN VITRO AND IN VIVO

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Mylotarg (Gemtuzumab Ozogamicin) is a humanised anti-CD33 antibody conjugated to the anti-cancer agent calicheamicin and approved for the treatment of relapsed acute myeloid leukaemia (AML). It induces cell cycle arrest and/or apoptosis of AML cells in vitro (Amico et al. Blood 2003;101:4589). About 15% of adult and 10% of children acute lymphoblastic leukaemias (ALL) are also CD33+ and could therefore be targets for Mylotarg. We have therefore investigated the cytotoxic activity of Mylotarg on CD33+ ALL in vitro and in vivo. Five freshly isolated cases of ALL which expressed CD33 on 78-90% of the cells were obtained. They were cultured in medium containing GM-CSF, IL3 and SCF and Mylotarg was added at 1 to 100 ng/ml. [H]-thymidine uptake was measured at 48-66 hours. Mylotarg induced 55-95% inhibition of thymidine uptake at 10-100 ng/mL, suggesting that CD33+ ALL cells are sensitive to Mylotarg in vitro. In order to set up an in vivo model of CD33+ ALL, one of the cases of CD33+ ALL (ALL-2) was established as a cell line by in vivo passage in irradiated SCID mice. To test the in vivo activity of Mylotarg, passed ALL-2 cells were inoculated in the tail vein of SCID mice at a dose of 5×10^6 cells/animal. These cells homed and expanded in the bone marrow, spleen and liver, as determined at different times by immunophenotypic analysis of bone marrow, spleen and liver using Mabs specific for human CD45 and CD33. Indeed at week 5 following tumour cell inoculation, ALL-2 cells represented a mean of 70% of bone marrow, 61% of spleen and 69% of liver cells. All animal died within 40-50 days following leukemia cell inoculation. To test the therapeutic activity of Mylotarg, 50 or 100 µg immunotoxin was inoculated i.p. on days 7, 11 and 15 following tumour cell inoculation. Injection of Mylotarg dramatically inhibited expansion of ALL-2 cells in all tested organs, including bone marrow. Indeed a mean of 1.5% and <0.5% CD45+ cells could be detected at week 5 in the bone marrow of animals treated with 50 and 100 µg Mylotarg respectively. Furthermore Mylotarg treatment increased survival of tumour injected animals by 22-40 days relative to controls. These data demonstrate that Mylotarg is active both in vitro and in vivo against CD33+ ALL cells.

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**CO026**
DAUNOXOME AND ARA-C AS REINDUCTION CHEMOTHERAPY IN RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA. HIGH COMPLETE REMISSION RATE DESPITE MULTIDRUG RESISTANCE OVEREXPRESSION

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Introduction. The treatment of relapsed or refractory acute lymphoblastic leukaemia (ALL) is frequently unsuccessful with current chemotherapy regimens and often there is an overexpression of multidrug resistance (MDR)-related proteins. Liposomal encapsulation makes daunorubicin less sensitive to the efflux effect of P-glycoprotein (PGP) and the in vitro data indicate that daunoxome (DNX) is more cytotoxic than daunorubicin against lymphoblastic leukaemia cell lines. In this study we assessed the efficacy and toxicity of liposomal encapsulated daunorubicin (daunoxome) plus cytarabine (Ara-C) as reinduction chemotherapy in high risk relapsed ALL patients (pts). The expression of MDR-related proteins (PGP, MRP, LRP) was also analyzed. Patients. 23 relapsed ALL patients; 5 ALL T, 18 ALL B (Smlg + 4/18), median age 36 yr (range 17-58), WBC (>10^9/L) 37 (range 4-222), Hb (g/L) 115 (range 50-146), Ph+ 8/23 (35%), Bcr-Abl + 9/23 (39%). More than 50% of pts overexpressed MDR at diagnosis and at relapse (pre DNX). 16/23 (70%) of pts were at first relapse and 7/23 (30%) were at second or subsequent relapse; seven pts (30%) relapsed after an allogenic BMT. All pts had received prior therapy with antracyclines, with a median cumulative dose of 212 mg/m² (range 55-445). DNX was given at the dose of 80 mg/m²/day (days 1-3) in 10/23 pts (43%) and at the dose of 100 mg/m²/day (days 1-3) in 13/23 (67%) of cases. In all pts Ara-C 2 g/m² (days 1-5) was given. Median number of cycles/pt: 1.2 (range 1-3). Results. 19/23 (83%) pts achieved a complete remission (CR) and 2/3 (9%) entered a partial remission (marrow blasts between 5 and 10%) for an overall response rate of 93% (21/23) with a tolerable toxicity and without cardiotoxicity. G-CSF was administered in 56% of cases, median days 7 (range 4-23). The median time to granulocyte (>0.5×10^9) and platelet (>2×10^10) recovery was 23 days (range 17-34) and 28 days (range 18-36) respectively. Mucositis (grade II-III WHO) was observed in 8
pts (34%) and Bacteremia was documented in 12/23 (52%) of cases; only one pts experienced mycosis. The early mortality was low (1 pts). Follow-up: 14/23 (61%) pts relapsed and 9/23 (39%) remain in CR; the median disease free survival (DFS) after DNX was 4.5 months (range 1-39) and the estimated 1-year survival rate was 37%. Conclusions. (i) Despite the small number of pts these data underline the high efficacy (83% CR rate) of DNX + Ara-C as reinduction therapy in relapsed ALL. Co-administration of G-CSF might reduce toxicity by improving granulocyte recovery; DNX dose intensification should be exploited to improve the RFS and OS. (ii) These results also confirm that DNX can overcome PGP-mediated drug resistance in ALL; in fact in our pts the response rate was not affected by the overexpression of MDR-related proteins. (iii) The high remission rate and the good tolerance in relapsed ALL pts suggest a possible role of DNX in front line ALL chemotherapy regimens.

CO027

RETROSPECTIVE PROGNOSTIC ANALYSIS REVEALS SIGNIFICANT SURVIVAL IMPROVEMENT IN INTERMEDIATE/HIGH-RISK GROUP: A SURVEY ON 467 ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) PATIENTS ENROLLED IN SUBSEQUENT "NILG" (NORTHERN ITALY LEUKAEMIA GROUP) TRIALS BETWEEN 1979-1999

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Starting 1979, 6 consecutive open phase II trials concerning adult ALL were activated at NILG sites (1979: HEAVD; 1984: OPAL-HD-ARA-C; 1987: reinforced HEAVD; 1991: IVAP; 1993: 07/93; 1996: 08/96). Overall, 467 patients with newly diagnosed ALL were enrolled in these studies (median age 33 years, range 13-74 years, median blast count >50x10^9/L, and range 0-999x10^9/L). Although different risk criteria were set for the conduct and the analysis of each of these trials, the aim of the present retrospective survey was the identification of a simplified prognostic model for overall survival (OS) that might document any significant survival improvement achieved with time in different risk categories. To this purpose some robust, dichotomized and reproducible prognostic indicators were chosen for inclusion into analysis: patient age and gender (25 to 60 years, with 5-year increments), FAB morphology, ALL immunophenotype (B-mature, B-precursor CD10 and CD10, T-cell), absolute blast cell count >5x10^9/L (10 to 100, with increments of 5-10x10^9/L), cytogenetics and molecular genetics (t(9;22)/BCR-ABL, t(4;11), t(1;19), others). The achievement of complete remission was excluded from analysis because obviously associated with longer survival. OS probability for the entire cohort of 467 patients was 0.26 10-20 years after the date of diagnosis. In subgroup analyses, patients without a known status for some variables (i.e. immunophenotype and cytogenetics) were excluded. The final model identified three major adverse risk factors for OS: (1) age >35 years, (2) absolute blast cell count >50x10^9/L, and (3) t(9;22)/BCR-ABL disease. On this basis, three different risk groups could be identified: the very high-risk group (VHR, i.e. all the cases with t(9,22)/BCR-ABL+ ALL; n=78), with a probability of OS at 10 years of 0.05; the intermediate/high-risk group (IHR, i.e. all those with t(9,22)/BCR-ABL- ALL and either age >35 years and/or absolute blast count >50x10^9/L; n=105), with an OS of 0.20; and the standard-risk group (SR, i.e. all those with t(9,22)/BCR-ABL- ALL and either age <35 years and/or a blast count <50x10^9/L; n=89), with an OS of 0.41 (p<0.01). To test the influx of treatment period, i.e. protocol (reflecting advances in general patient management as well as in techniques of disease control), OS of the three major risk groups was examined in relation to the dates shown above (before vs. after). Results did not evidentiate any change over time in OS rates of SR and VHR groups. Instead, 5-year OS rate of IHR patients was significantly improved since 1996, hence following the introduction of risk-oriented protocol 08/96 (<1996, n=64, OS 0.20 vs. 1996>, n=41, OS 0.40; p<0.01). This study documents a trend to improved OS results in adult ALL in recent years, in patients with IHR features. Although therapeutic progress occurs slowly, the role of risk factors and risk-adapted strategies must be adequately dealt with in ongoing clinical trials.

CO028

SINGLE HIGH DOSE IDARUBICIN AND HDARA-C (GIMEMA ALL-RESCUE 97) FOLLOWED BY STEM CELL TRANSPLANT FOR PRIMARY REFRACTORY OR RELAPSED ACUTE LYMPHOBLASTIC LEUKEMIA ADULT PATIENTS


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Outcome of adult patients with acute lymphoblastic leukemia (ALL) is discouraging, only about 30% of them becoming long-survivors, because early or late relapses are the main obstacle for the cure from leukemia. The GIMEMA group designed a phase II trial for adult ALL patients with refractory or relapsed disease. The salvage strategy included a single high dose idarubicin (IDA) combined with high dose cytarabine (ARA-C) (IDA 40 mg/m² on d 3, ARA-C 3 g/m² d 1 to 5), followed by a consolidation therapy including vindesine, high-dose methotrexate and dexamethasone, and then by a stem cell transplant (SCT) procedure, which was one of the main endpoints of the study. From 1998 to 2002, 135 patients from 25 Institutions were enrolled in the trial. 

Median age was 30 years, male/female ratio was 74/61. B-ALL or T-ALL was documented in 98 and 37 patients, respectively. Twenty-eight patients entered the study because primary refractory whereas 107 patients had had hematologic or extrahematological relapse. The Ph chromosome was documented in 21 cases, t(4;11) in 8, t(8;14) in 2. Complete remission (CR) had hematologic or extrahematological relapse. The Ph chromosome was documented in 21 cases, t(4;11) in 8, t(8;14) in 2. Complete remission (CR) was achieved in 75 patients (55%), including 11 Ph+ cases; 44 patients showed leukemia persistence and 16 died during reinduction. Forty-nine patients received SCT: 19 from a HL-A identical sibling, 15 from unrelated donors, 7 from a haploidentical relative, 2 cord blood, and 6 autotransplants. TRM was significant (26.5%), mainly affecting those receiving haploidentical SCT. Post-transplant relapse rate was also relevant (53%). Median disease free and overall survival were short (5.0 and 6.4 months, respectively); however, after a median follow-up of 20 months, 17 patients are alive, 11 of them disease free and 6 surviving with leukemia. Ten out of eleven leukemia-free survivors had received an allogeneic transplant: 6 from a HLA identical sibling and 4 from MUD. We conclude that this treatment induced a high CR rate in poor prognosis patients, thus rendering feasible a transplant procedure in most of them. However, significant rates of transplant-related mortality and post-transplant relapse point out to the search for more effective and less toxic conditioning regimens.

**CO029**

**SMALL AMOUNTS OF THE BCR/ABL P210 TRANSCRIPT ARE ALWAYS PRESENT IN PATIENTS WITH PH+ ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AT ONSET OF DISEASE**

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The BCR/ABL fusion gene, originating from the t(9;22) translocation, plays a key-role in the pathogenesis of Ph+ve leukemias. Three different breakpoint cluster regions (bcr) are recognized within the BCR gene, that are identified as major, minor and micro (M-bcr; m-bcr, µ-bcr), respectively. Due to the variable breakpoint location and to the promiscuous alternative splicing mechanisms three different BCR/ABL proteins are translated, sized 190kD (p190), 210kD (p210), 230kD (p230), respectively. Several in vivo and in vitro evidences suggested that the different BCR/ABL isoforms affect the leukemic phenotype. However, sensitive and specific techniques have recently shown that small amounts of p190 were detectable in almost all the patients with chronic myeloid leukemia. Therefore, in this study, using the accurate Real Time RT-PCR (Q-RT-PCR) assay, we evaluated the presence of the p210 fusion transcript in the diagnostic BM samples of ten ALL patients which resulted p190+ve p210-ve at the qualitative RT-PCR reaction. All patients were enrolled in the Italian Multicentric GIMEMA protocol for ALL treatment, six were females and four males, the patients mean age was 46.1 yrs (ranging from 32 to 66 yrs). Qualitative assay was performed according to the multiplex RT-PCR method (Haematologica 2003;88:275-9) that showed a sensitivity level of 1×10⁻⁴ and 1×10⁻⁶ for p190 and p210, respectively. Q-RT-PCR was performed according to the method described by the Europe Against Cancer Program for the standardization and quality control studies of Q-RT-PCR in leukemia. The Q-RT-PCR confirmed the p190 positivity in all cases with a diagnostic normalized mean copy number of 17×10¹ /ABL (ranging from 7.8 to 35×10⁻²). In addition, the Q-RT-PCR showed small amounts of p210 fusion transcript in all the ten patients with a normalized mean copy number of 6 /ABL (ranging from 1 to 10). The break in the m-bcr was confirmed by the FISH analysis with the ES dual colour translocation probe (Vysis-Abbott) that showed a dual fusion signal in the metaphases of the case tested. These results demonstrated the simultaneous occurrence of the p210 and p190 BCR/ABL isoforms at the onset of p190+ve ALL. Further studies will be needed to clarify the pathogenetic meaning of these evidences.
To define the clinical value of the real time quantitative reverse transcriptase-polymerase chain reaction (Q-RT-PCR) and of the qualitative nested RT-PCR assay in the monitoring of the ALL1/AF4 fusion transcript during patient clinical follow-up, we retrospectively compared the two methods to evaluate 18 ALL1/AF4+ve patients with acute lymphoblastic leukemia (ALL). Q-RT-PCR was performed according to the method described by the ‘Europe Against Cancer Program for the standardization and quality control studies of Q-RT-PCR in leukemia’. Eight patients were females, 10 males. Two patients were infants (< 1 year) and 16 were adults (median age: 37.5; range: 15-58 yrs). All cases presented an immunophenotypic pro-B ALL and received intensive conventional polichemotherapies. In one case autologous hemopoietic stem-cells were transplanted in first CR. Qualitative RT-PCR documented the ALL1-AF4 junction in all the 18 cases. By contrast, Q-RT-PCR amplified the ALL1/AF4 fusion in 17/18 (95%) cases, which showed at diagnosis a mean of normalised ALL1/AF4 copies of 5.9×10³/ABL (range 0.4 - 22×10³/ABL). In the remaining case, the nucleotidic sequence of the qualitative RT-PCR product showed the rare e10-e6 ALL1/AF4 junction, that fell outside the primer locations designed in the Q-RT-PCR protocol. After the induction treatment all patients achieved an hematologic CR. 12/17 patients also achieved a molecular CR (i.e. conversion to a negative qualitative nested RT-PCR status) while 5 persisted RT-PCR positive. These results were all confirmed by the Q-RT-PCR analysis. In particular, in the group of the 5 patients with molecular persistent disease, Q-RT-PCR showed an average reduction of < 1 log respect to the normalised ALL1/AF4 copies number detected at diagnosis. Afterward, all the 5 patients presented an overt clinical relapse, that was paralleled by a slight increase in the ALL1/AF4 copies that, however, remained inferior to the diagnostic value (8134×10³/ABL vs 1754×10³/ABL normalised ALL1/AF4 number of copies). In the group of the 12 molecular-CR patients we retrospectively compared 50 tests (mean 4.2 test for each patient; range 1 - 10). Concordant results were achieved in 48/50 (96%) tests. These latter were concordantly negative in 46 assays and concordantly positive in two cases. The two discordant tests concerned, in one case, the control at four years from diagnosis in a long survivors in which, in contrast to the negative qualitative PCR, Q-RT-PCR showed the presence of 27/ABL ALL1/AF4 normalised copies. This patient returned to a negative qualitative and Q-RT-PCR status at the following two controls while the patient follow-up has now reached the 5 years. In the second case a Q-RT-PCR positivity was documented at 6 months from diagnosis (9/ABL ALL1/AF4 normalised copies). The patient resulted negative at the following control but converted to both qualitative and quantitative positive status after one month and relapsed 8 months later. These data showed that nested qualitative RT-PCR and Q-RT-PCR have a similar behaviour respect to the monitoring of minimal residual disease in ALL1/AF4+ve ALL setting. However, in this study Q-RT-PCR resulted less specific than qualitative RT-PCR, failing to detect the ALL1/AF4 fusion in 1 of the 18 cases.
HEMOSTASIS AND THROMBOSIS

CO031 TREATMENT OF ACQUIRED HEMOPHILIA (AH) WITH RECOMBINANT ACTIVATED FACTOR VII (rFVIIa): DATA FROM THE ITALIAN REGISTRY

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The AH is a rare syndrome characterized by the sudden onset of bleeding, usually severe, in patients (pts) with a negative family or personal history of hemorrhages. Different therapeutic modalities have been used to control bleeding: human or porcine FVIII, DDAVP, high dose immunoglobulins, activated prothrombin concentrate, immunoadsorption and recently recombinant activated factor VII (rFVIIa). The available studies included small number of pts and carried out without controls; a control study in this clinical setting is unlikely to be carried out. rFVIIa has represented a major advance in the treatment of bleeding in congenital hemophilia with inhibitors. Its use in AH, details of administration, efficacy and safety are poorly defined although favourable reports in pts who failed other treatments suggest as first-line therapy (Hay CRM, Thromb Haemost 1997; 78:1463). We report on the use of rFVIIa in 15 out of 28 new pts with AH reported in the Italian registry in 2001. Two pts did not require treatment. 14 pts (20 bleeding episodes) received rFVIIa as first-line therapy, selected because the severity of bleeding: (mean ±SD) Hb g/dL 7.9 (1.2), RBC units 5.5 (0.6) and 1 after failure of DDAVP and porcine FVIII. 17 bleeds occurred spontaneously, 2 induced by intramuscular and intra venous injection, 1 after trauma. Three pts were treated as coverage of procedures (mastectomy, hemotoxar drainage, CVC insertion 1 each). Eleven patients received other first line therapeutic modalities. The clinical response was evaluated by the attending physician at the following intervals: <6, 12, 24 and >24 hours. Any adverse events occurring within 2 months of the end of treatment were registered. rFVIIa treatment (median range). One pt only received concomitantly tranexamic acid (Table 1). Bleeding was controlled in 13/15 pts (86.6%) and in 18/20 bleeding episodes (90.0%) in less than 24 hours without difference between intermittent or continuous infusion. Treatment was scored partial effective in 1 pt; 2 RBC units were transfused in the following 2 days. One bleeding related death occurred in spite of successive treatment with rFVIIa and high FVIII doses. The surgical procedures were uneventful. Bleeding recurred spontaneously in 4 pts (2, 3, 12 and 30 days after discontinuation of rFVIIa respectively) in the same site (3 pts) and in the pleural space in the pt in whom the thoracentesis was carried out. Four deaths unrelated to bleeding occurred. Two pts died because of the underlying neoplasia (breast cancer and myelodysplasia); 2 pts with pre-existing coronary heart disease and by-pass surgery, one died of myocardial infarction, the second of ventricular fibrillation 40 and 9 days after discontinuation of rFVIIa respectively. No thromboembolic events were registered during or in the immediate period after treatment. These data indicate the efficacy and the on treatment safety of rFVIIa in HA.

| Patients | 6 | 7 |
| Number of treatment | 11 | 12 |
| Initial dose (micrograms/kg) | 96 (40-118) | 92 (75-102) |
| Successive doses (micrograms/kg) | 90 (50-118) | 15 (8-10)* |
| Time interval (hours) | 3-6 | — |
| Number of doses | 9 (3-25) | 9 (3-25) |
| Days of treatment | 2 (0-7) | 5 (1-3-24) |
| Total dose (mg/treatment) | 36 (4.8-240) | 116.8 (45-254) |

*µg/kg/h

CO032 PROCOAGULANT ACTIVITIES (PCA) OF HUMAN ACUTE PROMYELOCYTIC AND BREAST CANCER CELLS ARE MODULATED BY ARSENIC TRIOXIDE (AS2O3) AND ALL-TRANS-RETINOIC ACID (ATRA)

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Remission induction of APL with ATRA or As2O3 is associated with a rapid resolution of the coagulopathy. Consistent with that, in APL cells, a reduction of the two major procoagulants (i.e. tissue factor (TF) and cancer procoagulant (CP)) is induced by ATRA and, limited to TF, also by As2O3. No information is available on CP sensitivity to As2O3. Further, ATRA controls the hypercoagulable state of breast cancer patients and both ATRA and As2O3 have anti-proliferative and proapoptotic activity in vitro against human breast cancer cells. Their effects on the procoagulants of these cells in vitro is not yet defined. Aim of this study was: 1. to characterize the effects of ATRA and As2O3 on CP in parallel to TF expression in APL cells and breast cancer cells; and 2. to investigate whether the modulation of the two procoagulants may be associated with drug-related apoptotic and/or differentiating effects. APL NB4 and breast cancer MCF-7 cell lines were incubated for 24h with 0.1 µM As2O3 or 1 µM ATRA or the combination of the two
drugs. Cells were then tested for CP activity, TF activity and antigen, apoptosis, differentiation and cell proliferation. The results showed that in NB4 cells, treatment with 0.1 µM As2O3 significantly reduced the to the same extent CP and TF activities (23% inhibition vs control, p<0.05), though less effectively than 1 µM ATRA (62% inhib., p<0.05) or As2O3/ATRA combination (65% inhib., p<0.05). In these cells, ATRA both alone and in combination with As2O3 significantly induced cell differentiation, evaluated as increased expression of cell membrane CD11b antigen, and apoptosis, whereas As2O3 did not. In MCF-7 cells, As2O3 significantly reduced both TF (38% inhib.) and CP (39% inhib.) activities (vs controls: p<0.01), however it was less effective than ATRA on TF reduction (67% inhib.), and equally effective on CP (40% inhib.). The As2O3/ATRA combination was as effective as ATRA alone. These data indicate that, both ATRA and As2O3 can affect PCA (CP and TF) in APL as well as breast cancer cells, although ATRA shows a greater effect than As2O3 on TF (in both cell types) and on CP (in NB4 cells). In APL cells, modulation of procoagulants by ATRA, but not by As2O3, occurred in parallel with the induction of differentiation and apoptosis. Differently, in MCF-7 cells procoagulant reduction always occurred in the absence of apoptosis. These results suggest a role for these two antitumor drugs, in the regulation of the blood clotting activation typically associated to leukemias and solid tumors, through the control of malignant cell procoagulant potential.

CO033
THE ITALIAN HAEMOPHILIA B MUTATION DATABASE
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In 2001 a national project, funded by the Italian Ministry of Health started with the scope of the identification of the causative mutation of all patients with hemophilia B in Italy. The project wanted to address two main problems: first to built a mutation database for timely carrier state and prenatal diagnosis and second to increase the knowledge of the genotype-phenotype relationship (inhibitors, anaphylaxis, severe-mild disease etc.). To date this project has involved 22 Italian hemophilia Centres (20 Centres sent us blood samples while Naples and Genoa sent us the mutations of 3 patients previously identified). 263 patients (nearly 70% of the estimated hemophilia B cohort in Italy) have been enrolled and 220 were not-related, but the enrollment is still ongoing. The FIX gene was screened in our laboratory by conformation sensitive gel electrophoresis (CSGE) which is based on the amplification of all exons and flanking intronic regions, the promoter and the region spanning the polyadenilation site by 4 multiplex and 2 single PCR. The mutation analysis have revealed 117 diverse mutations: 5 large gene deletions (3 complete and 2 partial), 10 small deletions and 102 single nucleotide substitution (74 missense, 15 nonsense, 10 in splicing sites and 3 in the promoter region). 30 mutations are present in different not related patients and we are exploring by linkage analysis (to date 94 out of 116 performed) the possibility of a founder effect (to date excluded in 34 and non excluded in 60). 17 substitutions involve CpG sites, which is considered an hot spot for mutations, and they are all previously reported into the international HB database (www.kcl.ac.uk/ip/petergreen/haemBdatabase.html). Two out of 4 patients with inhibitors show nonsense mutation, while the third shows a complete gene deletion (Bernardi et al., J Med Gen 1985) and the fourth a multi domain gene deletion (Hassan et al., Blood 1985). The rare presence of large FIX gene deletion in our cohort seems in keeping with the lack of cases of anaphylaxis following replacement therapy in Italy.

CO034
RISK FACTORS FOR THROMBOSIS IN PATIENTS WITH NEPHROTIC SYNDROME
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Background. Arterial and venous thrombosis are frequent complications in patients with the nephrotic syndrome (NS). Although some abnormalities of the hemostatic system have been described, the causes for the high thrombotic risk in these patients are still unclear. The aim of the study was to investigate the prevalence of some well-established thrombosis risk factors in patients with NS. Methods. The study included 84 patients with NS (M/F 41/43, median age 61 years) and 84 age- and gender-matched healthy subjects (M/F 41/43, median age 59 years). The following variables were investigated: the plasma levels of total homocysteine (tHcy), protein C, protein S, antithrombin (AT), fibrinogen, factor VIII, vitamin B6 and the serum levels of creatinine, albumin, folate, vitamin B12, cholesterol, the anticoagulant response to activated protein C (APC); glomerular filtration rate (GFR). Results. tHcy was significantly higher (12 vs 9 µM/L, p<0.001) and the anticoagulant response to APC significantly lower (APC
Whether inherited thrombophilia is associated with an increased risk for fetal loss (FL) is debated. We investigated 180 women with a history of unexplained FL (at least 2 FL or 1 late FL). The clinical records of 571 pregnancies were reviewed (median number 3, range 1 to 9); 117 viable infants were born to 82 subjects. Eighty-six women had 2 FL, 64 had more than 2 FL and 24 had more than 3 FL; 124 women had only miscarriage (defined as intrauterine fetal death before the 20th week of gestation), 30 had only late FL (defined as intrauterine fetal death after the 20th week of pregnancy), and 26 suffered from both circumstances. Sixty women had never been pregnant before 35 years of age. Out of the 180 cases, 31 women had a history of venous thromboembolism (VTE), 9 had a history of severe preeclampsia, and 2 had both VTE and preeclampsia. The control group consisted of 136 women older than 40 years, who had been pregnant at least once and who had no history of obstetric complications or vascular disease. All individuals were genotyped for the presence of factor V Leiden (FV-GA) and the G20210A polymorphism in the prothrombin gene (PT-GA). Moreover all the patients underwent a diagnostic panel including measurement of antithrombin (AT), protein C (PC), protein S (PS), and antiphospholipid antibodies (APA). Among the controls 2 individuals were heterozygous for FV-GA (1.5%) and 4 were heterozygous for PT-GA (3%). Among the overall patients 83 (46%) had thrombophilia (3 AT deficiency, 1 PC deficiency, 15 PS deficiency, 12 heterozygosity for FV-GA, 8 heterozygosity for PT-GA, 10 APA, 14 multiple abnormalities); inherited thrombophilia was present in 53 women (29%). After adjustment for other inherited or acquired risk factors, heterozygosity for FV-GA was found associated with an increased risk for overall FL (Odds Ratio 8.1, 95% CI 1.8 - 37.2), as well as for recurrent FL (OR 6.8, 95% CI 1.3 - 36.1) among the women with 2 FL, OR 7.6, 95% CI 1.3 - 43.5 among the women with more than 2 FL) and among the 56 women with at least 1 late FL (OR 5.4, 95% CI 1.6 - 18.2). The risk rose up to 25.0 (95% CI 4.4 - 141.9) among the 30 women with only 1 late FL, to 11.8-fold (95% CI 2.0 - 68.5) among the 42 women with multiple manifestations and to 11.2-fold (95% CI 2.1 - 60.7) among the 60 women older than 35 years at their first pregnancy. The risk associated with heterozygosity for PT-GA was significant only among the women with more than 2 FL (OR 4.8, 95% CI 1.2 - 18.8) and among the women with at least 1 late FL (OR 6.8, 95% CI 1.7 - 27.1). No increase in risk associated with FV-GA or PT-GA was found among the 24 women with more than 3 FL, in whom mechanisms other than the vascular ones could play a role. In conclusion the common polymorphisms FV-GA and PT-GA are associated with an increased risk for recurrent unexplained FL or late FL.
CO036
INCREASED RISK FOR DEEP VEIN THROMBOSIS IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) TREATED WITH COMBINED THALIDOMIDE-DEXAMETHASONE THERAPY
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In 2002, we started a phase II study with thalidomide and dexamethasone as first-line induction of remission for previously untreated patients with MM. The starting dose of thalidomide was 100 mg/d, with a subsequent increase to 200 mg/d after 14 days; the monthly dose of dexamethasone was 40 mg/d on days 1 to 4, with courses repeated on days 9 to 12 and 17 to 20 on the first and third month of therapy. Among the first 19 patients who entered the study (group A), 5 (26%) had symptomatic deep vein thrombosis (DVT) in the lower extremities. Based on this unexpectedly high frequency of DVT, treatment protocol was amended and the use of prophylactic fixed low-dose warfarin (1.25 mg/day) was instituted. Forty-three consecutive patients entered the amended study (group B); after a median of 4 months of thalidomide-dexamethasone therapy, DVT was documented by doppler ultrasonography in 4 (9%) of these patients. Overall, the risk for DVT in the entire series of patients was 14.5% and most of the patients had thrombosis within the first 2 months from the start of thalidomide therapy. Comparison between patients in groups A and B revealed that they were well balanced with respect to the main presenting features of MM, including abnormalities of chromosome 11 and 13. Baseline laboratory evaluation for risk factors for thrombosis - including antithrombin III deficiency, protein C and protein S deficiencies, resistance to activated protein C, lupus anticoagulant and antiphospholipid antibodies, prothrombin gene abnormalities (G20210A) - documented the presence of primary hypercoagulable states in 15% of patients in group A and in 17% of patients in group B. None of the 5 patients with DVT in group A had prothrombotic abnormalities, whereas a single patient out of 4 in group B who had DVT was found to be heterozygous for factor V Leiden. It is concluded that in patients with de novo MM, primary therapy with combined thalidomide-dexamethasone carries an increased risk of DVT; in these patients the hypercoagulable state is generally not related to identifiable prothrombotic abnormalities. Further studies are needed to elucidate the pathogenetic mechanisms underlying DVT and to assess whether fixed low-dose warfarin may provide an effective prophylactic measure to reduce the risk of thrombosis.

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CHRONIC MYELOPROLIFERATIVE SYNDROMES AND ACUTE MYELOID LEUKEMIAS

CO037
A P210-DERIVED MULTIPLE PEPTIDE VACCINE (CMLVAXX100) INDUCES REDUCTION OF STABLE MINIMAL RESIDUAL DISEASE IN CHRONIC MYELOID LEUKEMIA PATIENTS DURING TREATMENT WITH IMATINIB OR α-INTERFERON

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P210-derived b3a2 breakpoint peptides have been shown to induce, via binding with putative HLA class I and class II molecules, peptide-specific T-cell responses that mediated antileukemic effects in vitro. Previously, vaccinations with 5 breakpoint peptides and QS-21 in 12 chronic phase chronic myeloid leukemia (CML) patients induced an encouraging peptide-specific T-cell response in vivo in 3/12 patients but no effect on the disease was documented. In order to improve vaccine immunogenicity and antileukemic effect, in a multicenter study we added low doses of GM-CSF to the 5 peptides and QS-21 (CMLVAX100), targeting patients with stable minimal residual disease. Inclusion criteria of the study included a b3a2-CML, a major cytogenetic response stable for at least 6 to 8 months during conventional treatment (imatinib or IFN-α) and at least one of the HLA class I and/or class II molecules with binding properties for the peptides of CMLVAX100. While maintaining conventional treatment, vaccine treatment plan included 6 vaccinations at 2 weeks interval and 2 boosts after 4 and 8 months after the last vaccination. Seventeen patients have so far entered the study, 11 of them during treatment with Imatinib and 6 with IFN-α. Twelve out 17 patients completed the first 6 vaccinations and their characteristics of HLA and disease status and tumor response after 3 and 6 vaccinations with CM LVAX100 are depicted in Table 1. Tolerability to CM L VAX100 was optimal, with patients experiencing at most little discomfort at the site of injection. With regard to specific immune response, in 10/10 patients with proper HLA class II molecules we documented an important peptide specific CD4+ proliferation after 6 vaccinations in vitro which correlated in 7 of them with a strong delayed hypersensitivity skin reaction (DTH) in vivo (Table 1). All 5 patients with proper HLA class I molecules were studied for peptide specific IFN-γ production as a surrogate for cytotoxic T cell response. Preliminary results showed a weak but consistent peptide-specific IFN-γ production in 2/5 patients. All 12 patients showed a progressive reduction of their minimal residual disease during vaccinations with 5/12 becoming complete cytogenetic responders and 2/12 reaching a qualitative negative RT-PCR status for the b3a2 transcript. In conclusion, CM LVAX100 induces in most of vaccinated patients a peptide-specific T cell response which appears followed by an anti-leukemic effect as documented by a consistent reduction of previously stable minimal residual disease. However, the synergistic role of this active specific immunotherapy, observed here for the first time, needs to be confirmed in a larger cohort of patients.

Table 1. Characteristics (HLA status, stability of disease in months and type of conventional treatment) and response after CM LVAX100 of the 12/17 evaluable patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Months of stable disease</th>
<th>HLA</th>
<th>% Ph+ (qualitative PCR pos/neg)</th>
<th>DTH</th>
<th>Pre +3</th>
<th>12MV</th>
<th>12MV +6 vacc.</th>
<th>OLVAX100</th>
<th>OLVAX100 boost +6 vacc.</th>
<th>OLVAX100 boost +6 vacc.</th>
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<td>1 SA</td>
<td>12 (IFN)</td>
<td>DR1 10% (pos) 8% (neg)   0 (neg)</td>
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<tr>
<td>2 CAU</td>
<td>2 (Imatinib)</td>
<td>DR1 40% (pos) n.d. 17% (pos)</td>
<td>27% + / -</td>
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<tr>
<td>3 CY</td>
<td>1 (Imatinib)</td>
<td>DR11, DR1 5% (pos) n.d. 4% (pos)</td>
<td>3% -</td>
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<tr>
<td>4 SN</td>
<td>1 (Imatinib)</td>
<td>DR1 2% (pos) 2% (n.d.) 1% (pos)</td>
<td>+ + +</td>
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<tr>
<td>5 BM</td>
<td>12 (Imatinib)</td>
<td>DR11 10% (pos) 7% (n.d.) 0 (pos)</td>
<td>+ + +</td>
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<td>6 FF</td>
<td>8 (Imatinib)</td>
<td>DR11, DR1 3% (pos) 2.5% (n.d.) 0 (pos)</td>
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<td>7 MA</td>
<td>10 (Imatinib)</td>
<td>DR11 10% (pos) 0 0 (pos)</td>
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<td>8 CG</td>
<td>6 (Imatinib)</td>
<td>DR1, DR1 0 0 (pos)</td>
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<td>9 LC</td>
<td>18 (Imatinib)</td>
<td>DR1, DR1 0 0 (pos)</td>
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<td>10 W</td>
<td>3 (Imatinib)</td>
<td>DR1 25% (pos) 11% (n.d.) 1% (n.d.)</td>
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<td>11 MV</td>
<td>24 (IFN)</td>
<td>DR1 17% (pos) n.d. 8% (pos)</td>
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<td>12 MV</td>
<td>13 (IFN)</td>
<td>DR1 7% (pos) n.d. 3%</td>
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CO038
MOLECULAR QUANTITATIVE MONITORING OF BCR-ABL AND MDR1 TRANSCRIPTS IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED BY STI571

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The tyrosine kinase inhibitor imatinib mesylate (STI571) has been recently reported to produce remarkable hematologic and cytogenetic responses. However, it is not clear how long this benefit persists and how many patients might lose initial cytogenetic and molecular responses. Real-time quantitative RT-PCR is increasingly used to monitor responses in chronic myeloid leukemia (CML); this technique is very specific and has a good sensitivity; consequently, it represents a good tool for the sequential molecular monitoring of these patients. In this study, we have serially monitored
BCR-ABL transcripts in 87 bone marrow samples from 29 CML patients treated by imatinib from 5 to 33 months (median of 11 months). Results were compared with those obtained from cytogenetic and qualitative RT-PCR assays. Twenty-seven (93%) patients achieved complete hematologic response by 3 months; the 2 remaining cases were patients treated with STI in AP. Data on the bone marrow cytogenetic response at 6 months were available for 21 patients: 15 (71%) achieved a complete response, one a major, one a minor cytogenetic response and four (19%) did not achieve any response. At 12 months, 15 patients were evaluable and eleven (73%) achieved complete response; major responses then amounted to 13%. After qualitative nested RT-PCR assays, fourteen (48.3%) patients became PCR-negative; all these patients achieved also complete hematologic and cytogenetic remissions. Fifteen patients remained PCR-positive; twelve of them achieved complete hematological response and four also a cytogenetic remission. Nevertheless, four patients converted back to the PCR-positivity after 33, 11, 9 and 12 months respectively. By real-time PCR technique nine of 29 patients (31%) achieved molecular remission; eight of them were negative also in qualitative RT-PCR. Four cases converted back to the PCR-positivity: in two of these cases the PCR-positivity detected by real-time PCR preceded the positivity in qualitative RT-PCR. Twenty patients remained persistently PCR-positive; nevertheless, the BCR-ABL/ABL ratio was variable among patients, with 11 patients showing a transcript decrease of 1 log. A trend (p=0.09) to a longer PFS was observed in cases with a ratio that decreased >1 log after 6 months of therapy. Median BCR-ABL/ABL ratio of 0.17% at 4 months correlated with a complete or major cytogenetic response; patients not responsive showed a median ratio of 1.3%, thus confirming that the early molecular response to imatinib predicts cytogenetic response in CML. A good correlation between quantitative and qualitative PCR (24/29 cases) was also detected: three patients were PCR-positive in real-time but negative in RT-PCR; in these cases quantitative monitoring showed a transcript reduction >2 log. After quantitative monitoring four patients converted to PCR-positivity and other four showed an increase of transcript about 1 log. None of them lost cytogenetic or hematological response. Because efflux pump mechanisms such as P-glycoprotein are described to be involved in the resistance to STI, in fifteen patients MDR1 mRNA levels were evaluated. In three of four cases showing a BCR-ABL/ABL ratio decrease, MDR1 concomitantly decreased; in the four stable patients also MDR1 transcript was stable, but in five of seven patients showing an increasing transcript of BCR-ABL, this increase was preceeded by the increase of the MDR1 transcript. The results of this study show that some patients may lose the initial molecular response to STI STI and that MDR1 could be responsible for the acquired resistance during the treatment.

CO039
HYPEREOSINOPHILIC SYNDROME TREATED WITH IMATINIB THERAPY: CLINICAL AND BIOLOGICAL ANALYSIS

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Idiopathic hypereosinophilic syndrome (HES) is a rare hematologic disorder characterized by persistent eosinophilia with organ involvement. HES is defined as a peripheral blood eosinophilia greater than 1500 cells/ml for longer than 6 months, absence of other apparent aetiologies for eosinophilia and signs and symptoms of organ involvement. HES may be a reactive condition or a chronic myeloproliferative disorder but scanty information is available concerning its cytogenetic profile. The most common abnormality is the t(5;12)(q33;p13), which fuses the ETV6/TEL gene to the platelet-derived growth factor receptor-β (PDGFRB), a receptor tyrosine kinase that maps to 5q33, the same locus of EOS gene. PDGFRB, like ABL, PDGFRα, and c-KIT, is one of the tyrosine kinases inhibited by imatinib. Recently, Cools et al. reported the involvement of PDGFRα in a fusion transcript with FIP1L1, in some patients responsive to imatinib. To assess the clinical anti-proliferative activity of imatinib, a clinical trial for HES patients has been designed and approved. We have studied 22 patients with HES, two patients with PD/MDS with eosinophilic disorder and three with systemic mastocytosis (SM). Bone marrow samples (for morphology, blast%, eos%, etc.) and for cytogenetic and molecular studies were collected baseline and during the follow up. We studied in all patients the fusion transcript FIP1L1-PDGFRα by RT-PCR. The three SM patients were also studied for c-KIT status by RT-PCR and sequencing of coding regions. One HES patients was found positive for FIP1L1-PDGFRα fusion transcript. The patient respond to imatinib (400 mg/daily) therapy reducing the eos% in PB and BM, obtaining morphological and molecular CR. Instead, no response to therapy was reported in five patients, FIP1L1-PDGFRα negative, enrolled into the protocol with sufficient follow up.

Funding: This work was supported by the Italian Association of Cancer Research (A.I.R.C.), by Italian C.N.R., by MURST 40% target projects on CML, by A.I.L. grants, and Fondazione del Monte di Bologna e Ravenna grant.
CO040

IN Volvement of NITRIC OXIDE IN FARNESYLTRANSFERASE INHIBITOR-MEDIATED APOPTOSIS IN CHRONIC MYELOID LEUKEMIA CELLS


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Despite the clear molecular target, the mechanism of action of farnesyltransferase inhibitors (FTIs) is not fully clarified. We investigated the effects of various FTIs (α,β-hydroxyfarnesyl-phosphonic acid, manumycin-A and SCH66336) on proliferation and apoptosis in chronic myeloid leukemia (CML), using LAMA cells and bone marrow cells from 40 patients with CML in chronic phase. SCH66336 was more toxic on equimolar basis (50% inhibitory concentration, IC50: mean 5 μM; range 1-10) compared to manumycin-A (IC50: mean 50 microM; range 25-75) and to α,β-hydroxyfarnesyl-phosphonic acid (IC50: mean 100 microM; range 75-150). In subsequent experiments with primary cells we used the mean IC50 concentration of each FTI established in LAMA cell line. Using methylcellulose colony assay, a dose dependent FTI-mediated cytotoxic effect was observed in LAMA cells and in 65% of primary CML cells, whereas bone marrow progenitor cells from healthy controls were only weakly affected by FTI exposure (mean inhibition of CFU±SEM: 47.7±7% vs 15.5±1%; p<0.001). This FTI-induced cytotoxic effect was in part related to enhanced apoptosis as detected by DNA fragmentation from total BM CML cells cultured in the presence of FTIs after extraction of low molecular weight DNA or flow cytometric DNA analysis following propidium iodide staining, and in situ terminal deoxynucleotidyl trans-ferase (TdT) assay. However, Fas signalling was not involved, as Fas-receptor (FasR) and Fas-ligand (FasL) expression were not modified by FTI exposure, the susceptibility to FTI-mediated inhibition did not correlate neither with FasR nor with FasL expression in CD34+ CML cells, and intracellular activation of caspase-1 and caspase-8 were not altered by FTIs. Since caspase-3 is the main executioner caspase required for apoptosis, we assessed the ability of FTIs to induce caspase-3 activation by flow cytometric measurement of the fluorescence generated after cleavage of the specific fluorogenic substrate DEVD-AMC. FTIs clearly increased fluorescence intensity, indicating that caspase-3 was activated: mean percentage of fluorescent CML cells treated with FTIs was 35±3% vs 5±1% in control cultures (mean of 5 experiments). In addition, pre-incubation of CML cells with the caspase-3 inhibitor Z-DEVD-FMK partially abrogated FTI-mediated caspase activation (mean%± SEM: 18±5%; p=0.03). FTI exposure did not modify the expression of bcl2, bclxL and bclxS. Using PCR and Western blotting, we demonstrated the presence of iNOS mRNA and protein in primary CML total BM cells. A much stronger amplification of iNOS signal was obtained after exposure of primary total BM CML and LAMA cells to FTIs, resulting in a higher intracellular NO production measured directly by flow cytometry using the cell-permeable fluorescent indicator DAF-2 DA. Moreover, inhibition of NO synthesis by the iNOS inhibitor NG-monomethyl-arginine (500 microM) partially abrogated the effects of FTIs on NO production and apoptosis. Furthermore, C3 exoenzyme, an Rho inhibitor, significantly increased iNOS expression in CML cells suggesting that FTI may up-regulate NO formation.

In conclusion, our results demonstrate that FTIs can induce apoptosis in CML cells and this is not accompanied by Fas-R/Fas-L or bcl2 modulation, but rather involves iNOS and caspase-3 activation at least partially through FTI-mediated inhibition of Rho.

CO041

ANAGRELIDE IN ESSENTIAL THROMBOCYTHEMIA: A RETROSPECTIVE STUDY


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Anagrelide is a platelet lowering drug very effective in ET and other chronic myeloproliferative disorders. This non mutagenic molecule is preferentially used in patients with long life-expectancy and its side effects are usually mild and permitting a long-lasting treatment. So far, there are no controlled data on the cardiovascular and hematologic toxicity and on the disease related symptoms and events. This retrospective study includes 180 patients from 26 Institutions of the Gruppo Italiano Malattie Mieloproliferative Croniche (GIMM C). The...
objective of the study is to evaluate the spontaneous policy on Anagrelide use in ET and to obtain new data on Anagrelide cost-effectiveness, in order to be able to design a proper prospective study. The patients, 66 males and 114 females (ratio 0.57), at diagnosis had a mean age of 39 years (age 13-40 years 57%, 41-60 years 31%, 61-82 years 12%) showed a mean PLT count (10^9/L) of 1129, previous thrombosis (9%), previous hemorrhage (3%), disease related symptoms (19%), cardiovascular risk factors (31%) and splenomegaly (29%). At start of Anagrelide treatment the mean PLT count (10^9/L) was 904 (below and over 1000 in 66% and 34%, respectively), 69% of patients were receiving an antiplatelet treatment and 70% of patients received a previous cytoreduction (Alkylating agents and HU 19%, HU alone 19%; HU and IFN 18%, IFN alone 14%). The treatment with Anagrelide was started mainly because of the young age of patients (60%), inefficacy and/or high dose required of the other drugs (38%), toxicity and/or side effects of the other drugs (24%) and patient request (13%). The mean Anagrelide dose from the baseline of 1 mg was increased to 1.6 mg (range 0.5-6.0) after one month and was 1.9 mg (range 0.7-3.5) after 48 months. The most common side effects were cephalea/vertigo (27%), palpitation/tachycardia (25%), gastrointestinal (24%), asthenia (11%), oedema (5%). A transitory interruption of Anagrelide treatment was observed in 34/180 cases (19%) as a consequence of drug toxicity (1.7%), side effects (5%), other causes (8.3%), no drug availability (4%). A drug withdrawal was registered in 54/180 patients (30%) after a median treatment duration of 17 months (when the median Anagrelide dose was 2 mg/day and the median PLT count was 556 x10^9/L), because of inefficacy (3.3%), toxicity (4.5%), side effects (13.5%), compliance loss (2.5%), other causes (4.2%), no drug availability (2%). The mean follow-up of all patients (n=180) and of the patients still on treatment (n=126) is 22 and 24 months, respectively. The mean PLT count (10^9/L) from the baseline value of 932 decreased to 607, 512, 491, 481 and 442 after 1, 6, 12, 36 and 60 months, respectively. The mean WBC count (10^9/L) from the baseline value of 932 decreased to 22 and 24 months, respectively. The mean PLT count (10^9/L) was 7.7 at the baseline and 9.1 after 60 months. The mean Hb level (g/dL) of 13.2 at the baseline decreased to 12.7 and 12.5 at months 6 and 60, respectively; moreover, Hb below 10 g/dL was registered at the baseline and at month 12 in 4% of cases, at month 36 in 8% and at month 60 in 12% of cases. Two minor hemorrhagic events (0.9/100 pt-yrs) and three major thrombotic events (0.9/100 pt-yrs) were observed during the follow-up. In this series of ET patients the treatment with the non-mutagenic Anagrelide has been confirmed to be effective and globally well tolerated. Nevertheless, new prospective studies are necessary to define the Anagrelide long-term toxicity, the incidence of thrombotic and/or hemorrhagic complications and the anemia occurrence.

Large deletions adjacent to the t(9;22) breakpoint on the derivative 9 chromosome have now been found, which may have a selective advantage for an emerging tumor cell as well as an increase in the size of the target cell population for mutagenesis. We have characterized the precise extension of the deletion on der(9) in 20 CML cases using fluorescence in situ hybridization (FISH) analysis with an appropriate set of BAC/PAC probes to attempt a better definition of TSGs encompassed by these genomic deletions. FISH analysis was performed on patients' bone marrow cells at diagnosis. Chromosome preparations were hybridized in situ with probes labeled with Cy3 and FluorX by nick translation. A mixture of PACs RP5-835J22 and RP5-1132H12 was used to identify the ABL gene; BAC RP11-164N13 encompasses the major BCR breakpoint region. A total of 35 probes for chromosome 9 and 13 probes for chromosome 22 were used. UCSC (University of California Santa Cruz, http://genome.ucsc.edu/) database was queried for probe location. Chromosome 9 deletions on the der(9) were detected in 15 (75%) cases. All the deletions were detected at diagnosis and were present in all Ph+ metaphases; the size of the deletion ranged from 350 Kb to 7.0 Mb. The TSG PTGES gene (prostaglandin E synthase) was lost in 11 (73%) cases. Chromosome 22 deletions on der(9) were found in 18 (90%) of the analysed cases, the size of the deletions ranging from 400 Kb to 3.5 Mb. Two TSGs were found located inside the deleted sequences of chromosome 22: SMARCB1 (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily b, member 1) and GSTT1 (glutathione S-transferase π1). These TSGs were found deleted in 16 (89%) cases bearing deletions of chromosome 22. Moreover, among the 20 patients with deletions, at least one of the above TSGs was deleted in 18 (90%) patients. Fourteen (70%) patients were treated with alpha-IFN ther-
apy: 12 did not obtain complete hematologic remission (CHR) and 2 were not evaluable for response. Therefore, 3 started Glivec in the blastic phase, resulting in a major cytogenetic response (MCR) in two cases and only HCR in the remaining one. The latter, together with another one, died of CML progression after about 1 year from the beginning of the Glivec treatment. Among the remaining 9 non responders to alpha-IFN therapy, 7 were treated with Glivec during the chronic phase, and all obtained CHR and MCR. The observation that deletions on der(9) are associated with the loss of TSGs suggests their possible involvement in the CML outcome, mediated by a haplo-insufficiency mechanism; the prognostic impact of such cytogenetic abnormalities in CML patients treated with Glivec should be further evaluated.

Molecular repositivization of the Bcl-2/IgH nested PCR assay is an uncommon but well-defined event among FL patients achieving molecular remission (MR) after autografting. This finding is regarded as a clear evidence of active disease and associated with a high risk of clinical relapse. We have recently observed on a large sample of patients that non-neoplastic Bcl-2 rearrangements are very rare in chemotreated patients during the first two years following chemotherapy. However it cannot be excluded that at least a proportion of Bcl-2/IgH repositivizations might be due to unrelated rearrangements and not to reappearance of the original FL clone. To investigate this possibility we analysed FL patients with Bcl-2/IgH repositivization by sequencing their rearrangements at diagnosis and at molecular recurrence. The latter was defined as reappearance of Bcl-2/IgH PCR positivity on two bone marrow samples obtained from patients that were previously considered in both continuous complete remission and MR (defined as PCR-negativity on two samples taken with at least a 3-months interval). We have so far analysed the molecular follow-up of 119 FL patients treated with autologous transplantation with or without Rituximab supplementation (103 vs 16). Of these, 75 achieved molecular remission during their disease history. Molecular recurrence following molecular remission was observed in 8 of 75 patients (10.6%). Median distance from transplantation to molecular recurrence was 11 months (range 7-106). In 6 patients the rearrangement detected at molecular relapse was identical to that observed at diagnosis. In 3 of them molecular recurrence was followed by overt clinical relapse after a median of 7 months (range 1-12). In contrast, in 2 patients the Bcl-2/IgH rearrangement detected at recurrence was unrelated to that observed at diagnosis (as defined by the presence of different breakpoints, N insertions and JH usage). Of note, PCR-positivity due to unrelated rearrangements occurred after several years from the end of treatment (38 and 106 months, respectively), as opposed to most of relapses occurring with the original FL rearrangement (median 10 months, range 7-87). Unre-
lated rearrangements appeared to be persistent in repeated molecular follow-up analyses. We are currently monitoring these patients from both the molecular and clinical point of view to detect any evidence of second lymphoma. So far all of them are in continuous clinical remission after a follow-up from molecular recurrence of 2 and 20 months. Based on these results we recommend confirmatory direct-sequencing analysis for patients reverting to PCR-positivity following a prolonged period of MR, to avoid inappropriate overtreatment of these subjects.

C0044
HIGH RESPONSE RATE AND LOW TOXICITY WITH AN INTENSIVE, SHORT-TERM CHEMOTHERAPY PROGRAM FOR BURKITT’S LYMPHOMA IN ADULTS
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We tested a very short and intensive chemotherapy program in a consecutive monoinstitutional group of 18 adult Burkitt’s lymphoma (BL) patients. The main patient characteristics were: median age: 35.5 years (range 18-68); Ann Arbor stage I-II/III-IV: 10/8; bulky disease: 13 pts; LDH ≥ 1000 U/mL: 8 pts; ECOG P.S. 0-1/2-4: 13/5. After 5-week cytoreductive chemotherapy consisting of vincristine (VCR), cyclophosphamide (CTX), doxorubicin (ADM), high-dose (HD) methotrexate (MTX), and intrathecal MTX or cytarabine (ARA-C), a consolidation phase including HD ARA-C plus cisplatin (CDDP) was provided as a 4-day continuous infusion. The median duration of the chemotherapy program was 63 days. Overall response rate was 88%. One toxic death was recorded during treatment in complete remission (CR). Four patients were treated with high-dose sequential (HDS) chemotherapy supported by a PCRNegative autologous peripheral blood stem cell (PBSC) transplant for relapsing or refractory disease: 3/4 are in CR after 16, 19, and 38.5 months; 1 died of progressive disease (PD). Overall survival (OS) and progression-free survival (PFS) were 88% (95% CI, 73% to 99%) and 77% (95% CI, 53% to 99%), respectively. Surviving patients have been followed for a minimum of 4 months to a maximum of 149 months. The treatment was well tolerated except for one toxic death after HD MTX and hematologic toxicity after HDARA-C/CDDP. In conclusion, this intensive chemotherapy program is the shortest schedule for disseminated adult BL and prevents severe adverse events. In addition, the HDS regimen supported by PCRNegative PBSC transplant proved active in refractory/relapsing patients and should be investigated as salvage therapy.

C0045
THE PROGNOSTIC ROLE OF TUMOR AMOUNT AND DISTRIBUTION IN Hodgkin’s Lymphoma

Tumor burden (TB) has been demonstrated to be a powerful prognostic factor in Hodgkin’s lymphoma (HL). TB measurement is now possible through the evaluation of all the lesions recognizable in total body CT scans. We investigated the clinical role of TB on a wide retrospective series of patients, in comparison with the majority of the current elementary prognostic factors and with the best composite indices. The volume of TB at diagnosis was directly measured on the initial staging CT scans of 351 HL patients who were diagnosed in different institutions and entered standard treatment protocols. Mean age was 34.0±16.4 years, males/female ratio 168/183. Clinical stage was I in 46 cases, II in 199, III in 64 and IV in 39. Overall (OS), disease-free (DFS), failure-free survival (FFS) were the time parameters analysed by means of the Kaplan and Meier’s technique and the Cox’s univariate and multivariate method applied to the proportional hazard model. A logistic regression was used related to the achieved or failed complete remission. The prognostic indexes of the International Prognostic Factor Project (IPFP), of the Memorial Sloan Kettering Cancer Center (MSK), of the International Database on Hodgkin’s Disease (IDHD), and the triply Integrated Index (II) were evaluated. The mean TB, normalized to body surface area (rTB), was 137.0±123.6 cm^2/m^2 (range: 1.9 - 694.5). At multivariate analysis for prognostic value rTB was the parameter that statistically correlated best with FFS and DFS followed by number of involved sites. RTB was the second prognostic factor for OS after age and the first predictor of complete remission after treatment. A prognostic model including rTB and number of involved sites demonstrated to fit the FFS distribution largely better than every other composite index analysed. In conclusion, rTB is a very strong prognostic factor in Hodgkin’s lymphoma, more powerful than and largely independent of those hitherto known and used. Such emerging primary prognostic importance of both tumor amount and distribution stimulate further studies to exploit their possible impact on the clinical management of patients.
CO046
FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINATION IN THE TREATMENT OF PATIENTS WITH INDOLENT NON-FOLLICULAR B-CELL NON-HODGKIN’S LYMPHOMA. PRELIMINARY RESULTS OF A PHASE II TRIAL BY THE "GRUPPO ITALIANO PER LO STUDIO DEI LINFOMI" (GIL)


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Introduction. Indolent non follicular lymphomas (INFL), comprise a rather heterogeneous subgroup of lymphomas, including small lymphocytic lymphoma (SLL), Immunocytoma (IC) and marginal zone lymphomas (MZL). With available treatments a median survival of 5 to 10 years is expected. Typical initial treatment strategies vary from the use of single agents to high doses therapies but alkylator single agent chemotherapy can still be considered as the standard treatment. In April 2002 we started a phase II trial aimed to verify the efficacy of Fludarabine and Cyclophosphamide (Flu-Cy) combination in this subset of NHL, in terms of response, survival and safety. Patients and methods: To be included in the trial patients should have a diagnosis of SLL, IC, MZL or CD5-ve mature B cell leukemia (MBCL), supported by morphologic, phenotypic and molecular data; patients should also be untreated for lymphoma and have active disease defined by the presence of anemia (Hb <11 g/dL), thrombocytopenia (Plt <100.000/m3), bulky disease, rapidly increasing lymphocytosis or enlarging masses. Treatment consisted of Fludarabine 25 mg/m² iv day 1-3 and cyclophosphamide 300mg/m² on IV days 1 to 3, to be repeated every 28 days for 6 cycles; an intermediate evaluation of response after 3 cycles was planned and an adequate anti-infective prophylaxis was mandatory: the use of G-CSF was not mandatory. Results: As of June 2003, 31 patients were registered into the trial; one patient was excluded from the study due to incorrect histology. Median age of the remaining 30 patients was 64 years (range 40-75), M/F ratio was 2.1. The diagnosis was SLL in 9 patients, IC in 1, MZL in 13 and MBC in 7. All patients had stage IV disease; 4 patients presented with B symptoms. Anemia (Hb <11 g/dL) was present in 27%, absolute lymphocytosis in 43%, ESR >30 mm in 39%, elevated β<sub>2</sub>-microglobulin in 60%, abnormal LDH in 29%. At the time of the present analysis 17 patients underwent intermediate evaluation after three cycles with 4 CR (24%) and 13 PR (76%); so far, only 11 patients completed the treatment program with 6 CR (55%) and 5 PRs (45%). Two patients died during treatment, one after the 2nd cycle due to erosive pulmonary aspergillosis, one due to bone marrow aplasia occurred after the 4th cycle. Overall, grade III or IV hematologic toxicity was observed in 4 patients. After a median follow up of 6 months only one patient died, due to lymphoma progression 4 months after treatment completion. Conclusion: The preliminary results of our study demonstrate that Flu-Cy combination is effective in the treatment of patient with INFL but has also shown a relevant toxicity profile. The final evaluation of the trial will allow to draw definitive conclusions on efficacy and safety profile of Flu-Cy regimen in this subset of NHL.

CO047
CHLVVP/ABVVP, A NEW HYBRID COMBINATION CHEMOTHERAPY FOR NEWLY DIAGNOSED ADVANCED HODGKIN’S DISEASE: CLINICAL RESULTS OF A RETROSPECTIVE ANALYSIS


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ABVD is considered the standard treatment for patients with advanced stage Hodgkin’s disease. However clinical results achieved with such chemotherapy regimen are so far to be evaluated as satisfactory. Since only 60-65% of patients can be considered cured with standard ABVD. In order to verify a possible improvement in clinical results, new chemotherapy regimens incorporating all of the active drugs are under investigation. We here reported our experience in advanced Hodgkin’s disease patients with a new hybrid CT regimen that included VP16 and is delivered with a weekly schedule. Sixty-one patients (35 males, 26 females) 31 years median age all diagnosed with advanced Hodgkin’s disease were retrospectively considered. All patients received Clofambucil 6 mg/m² days 1-7, VELBE 6 mg/m² days 1; Procarbazine 80 mg/m² orally day 1 - day 7; Prednisone 50 mg day 1- day 7; Adriamycin 30 mg/m² days 8-10; Vincristine 2 mg day 8; Bleomycin 15 mg day 8,15. All cycles were repeated every 3 weeks for a maximum of 6 cycles. Fifty-six out of 61 (92%) patients achieved CR, 2 (3%) patients achieved UCR, with ORR of 95%. Fourteen patients relapsed or progressed. With median follow-up of 5 years the proportion of patients without events is 76.6% (95 CI: 66.5-88.1), while the proportion of patients alive is 79.3% (95 CI: 68.5-91.3). The most relevant hematologic tox-
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There are over 100 autoimmune diseases which are clinically relevant, and current and also innovative therapies have produced little more than remissions but never cures. Leaving aside monoclonal antibodies targeting B cell subpopulations, there are now three new aggressive approaches for the treatment of severe autoimmune diseases (SADs): 1. High-dose cyclophosphamide (HDCY) without SC rescue (the John Hopkins paradigm), 2. High-dose immunosuppression (HDIS) followed by allogeneic SCT, and 3) HDIS followed by autologous SCT, most generally from the blood. ASCT, which originated from the classical van Bekkum’s and Ikehara’s animal experiments, was first proposed for intractable SLE by myself a decade ago, and is now currently performed worldwide. Numerous extensive reviews and a comprehensive book have been published. Here only three important experiences will be briefly reviewed. Phase I/II studies in multiple sclerosis (MS) have shown that transplantation may positively affect the disease by stabilising the clinical condition and by completely abrogating the immunophysiological process in the CNS as evidenced by MRI imaging. A phase III study known as ASTIMS is under way in Europe. Two phase I/II trials for systemic sclerosis (SSc) have shown an initial TRM of 17%, which subsequently fell to 12.5% and later still to 8.7%, while deaths related to disease progression accounted for 14%. These reports showed the durability of CR and/or PR in two thirds of the patients up to 3 years after ASCT. Fifty-three patients (27 male; age: 30-60, mean: 47 yrs) with different malignancies (6 HD, 13 NHL, 11 MM, 2 CML, 3 AML, 1 CLL, 3 Kidney and 5 Breast cancer) were grafted with unmanipulated PBSC from fully matched HLA-identical sibling. As conditioning regimens, patients received: 14 Flu/Cy, 15 Flu/TBI, 15 Flu/Mel. GVHD prophylaxis consisted of CSP/MTX. Chimerism analysis was performed using multiplex PCR amplification of short tandem repeat markers and fluorescence detection. Fifteen STR loci and the Amelogenin locus are co-amplified in a single reaction. Donor/recipient cell population ratio at different intervals was detected by calculating peak area of PCR products for each informative marker. The median number of informative alleles was 6 (range 3-9). Donor engraftment was evaluated on unfractionated peripheral blood (PB), bone marrow samples and on separated PB CD3+ cells after sorting with immunomagnetics beads (Mini-Macs, Miltenyi Biotec). Median follow-up for survival was 161 days (34-653). The rates of complete donor chimerism (CDC →95% donor cells) at day +15,+30,+90,+180,+270,+360 and beyond +360 were 23%, 36%, 60%, 86%, 93%, 93% and 93% respectively. Kinetics of engrafting donor cells was different in the three conditioning regimens Flu/Mel, Flu/TBI, Flu/Cy. The rate of CDC at days +30 were 73%, 20%, 0%, respectively; at days +90 were 92%, 57%, 0%, respectively. Full donor T cells engraftment preceded donor myeloid engraftment: at days +30 mixed chimerism →50% was detected on 33% and 78% of bone marrow and CD3+ cells samples analyzed respectively. Twenty-eight patients (64%) are alive to date. Conclusions: Our study

References


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VP-16 50 mg/m² was added in 7 pts transplanted from refusal, seven because disease progression. The BMT identified an UD. (Two pts have not been transplanted because relapse. 59 pts started the search for an unrelated patient, 47 with an HLA identical (IS), 1 syngenic and 1 5/6 allele, only 2 patients were HLA-matched, while all others were HLA-mismatched with their donors for 1 (n=14) or 2 (n=14) HLA antigens. The leukemia was considered as starting dates. With a median follow-up of 1151 days (range 44-5151) the overall survival (OS) of the 25 UD-HSCT pts was 44%, 44% for patients in early disease and 44% for patients in advanced disease. In the IS-HSCT group (49 pts), the OS is 43%, 65% for pts in early phase versus 10% in advanced phase (p=0.0003).The OS fails to 20% for the 34 evaluable patients who failed to undergo transplant (p<0.03). The different outcome in the UD HSCT and IS HSCT cohorts for advanced leukemia may be related to the fact that UD transplants were done in patients in less advanced phase than family transplants. The main causes of failure were TRM in the UD-HSCT group and disease progression in the others. In our experience the best therapeutic option for HRALL is allogeneic transplant with OS >40% while pts without a donor have a long-term OS of 20%. So we recommend initiating a search for an UD for all pts lacking a suitable family donor.

CO052
IDENTICAL, SINGLE CENTER POLICY FOR UNRELATED CORD BLOOD TRANSPLANT IN YOUNG PATIENTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA: A LONG-TERM FOLLOW-UP
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In the past decade, hematopoietic stem cell transplantation using cord blood graft (CBT) has increasingly been utilized, particularly for pediatric patients lacking a related HLA compatible donor. We report the long-term results of a prospective, single Center study on 30 consecutive pediatric and adolescent patients undergoing an unrelated CBT for high-risk acute lymphoblastic leukemia (ALL) between December 1995 and March 2003. Conditioning regimen, GVHD prophylaxis, infectious disease prevention and supportive therapy were identical for all patients. The median age was 10.5 years (range 5-20), the median body weight was 40 kg (range 13-80). After high resolution molecular typing of DRB1 allele, only 2 patients were HLA-matched, while all others were HLA-mismatched with their donors for 1 (n=14) or 2 (n=14) HLA antigens. The leukemia was considered at intermediate risk for 21 patients in 2nd complete remission (CR) at time of CBT and at high risk for 9 patients in more advanced stage. The conditioning regimen consisted of 12-Gy fractionated TBI, etoposide, cyclophosphamide and antilymphocyte globulin (ALG). As GVHD prophylaxis, all patients received cyclosporine and low-dose corticosteroids. The median number of nucleated cells (NC)/kg of the recipient b.w. was 3.8×10¹⁰ (range 1.1.56-11.3) at collection and 3.3×10¹⁰ (range 0.58-10.9) at infusion. The median proportion of NC lost after thawing was 8% (range 0-44). The median number of infused CD34+ cells>10⁶/kg of the recipient b.w. was 2.1 (range 0.2-8.9) and the median number of infused CFU-GM×10⁴/kg of the recipient b.w. was 1.6 (range 0.05-9.8). All but one of 26 evaluable patients achieved PMN count > 0.5×10⁹/L after transplant at a median of 31 days (range 20-73), with a probability of myeloid engraftment with full donor chimerism of 100% at 90 days. Grade II-IV and III-IV of acute GVHD occurred in 5 (17%) and 3 (10%) patients, respectively. Chronic extensive GVHD developed in 3 of 19 (16%) evaluable patients. At a median follow-up of 58 months (range 9-
87), 13 patients are alive and disease free with full donor chimerism. The actuarial probability of transplant related mortality (TRM) and relapse at 5 years was 28% (CI:12-44), and 42% (CI:22-62), respectively. For all patients, the advanced stage of disease was associated with higher risk of leukemic relapse (intermediate vs advanced stage: 36% vs 50%), but the difference was not statistically significant. The 5-year probabilities of overall survival (OS), leukemia-free survival (LFS) and event-free survival (EFS) were 38% (CI:19-57), 42% (CI:24-60) and 43% (CI:25-61), respectively. In univariate analysis the most important factor favorably affecting outcomes in terms of TRM, OS, LFS, EFS was higher dose of CFU-GM infused. In particular the EFS for patients receiving a dose of GFU-GM×10^4/kg<1 (n=8), >1<2 (n=11) or >2 (n=11) was 13%, 45% and 62%, respectively (p=0.02). Conclusions: CBT is a valid option for the treatment of high risk ALL young patients lacking an HLA compatible family donor. The cell dose expressed by the number of CFU-GM infused is highly predictive of outcomes and should be considered the main parameter in selecting a cord blood unit for transplant.

C0053
EFFICACY AND SAFETY OF DLI AFTER T DEPLETED BMT FOR THERAPY OF THE RELAPSE
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Thirty-eight patients (28 matched, 10 mismatched) were treated with DLI because of molecular (n=12), cytogenetic (n=15), hematologic (n=11) recurrence of their disease (24 CML, 3 multiple myeloma, 9 AML, 2 ALL). DLI was given at a median of 12 months (range 1-132) after T-cell depleted HSC transplant, on the basis of the following factors: disease, type of relapse, HLA disparity, interval between transplant and relapse. The number of infusion ranged from 1 to 30 (median 9) and the dose of each infusion ranged from 1×10^4/kg to 2×10^4/kg. Dose escalation was required in 23 patients to achieve a response. Of the 24 CML, 9 patients achieved molecular and 7 hematologic responses, all but 1 of 6 patients treated because a molecular relapse did not progress to cytogenetic or hematologic relapse. The median follow-up was 61 months. Disease progressed in 2 of 3 patients with multiple myeloma. The other achieved complete clinical and molecular remission at a follow-up of 49 months. 4 of the 9 patients in the DLI AML group relapsed, 3 achieved complete hematologic and molecular remission (median follow-up 29 months) and 2 with mixed chimerism and no residual disease remained stable (follow-up: 7 and 10 months). One of the two patients with ALL relapsed while the other achieved complete hematologic, clinical and molecular remission with a follow-up of 16 months. Complications included 1 case of grade II acute GVHD disease which resolved with ATG, steroids and cyclosporine therapy; 3 cases of grade I GVHD which resolved with steroids and 4 cases of chronic GVHD which was controlled with local steroid therapy. DLI after T-Cell depleted matched or mismatched transplant under our current protocol, is feasible and is not associated with major risks. The response rate is good particularly in CML and AML as remission was confirmed in long term follow-ups.

C0054
THE "BOLOGNA 96" CLINICAL TRIAL OF SINGLE VS. DOUBLE AUTOLOGOUS TRANSPLANTATION FOR PREVIOUSLY UNTREATED PATIENTS WITH MULTIPLE MYELOMA
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Between January 1996 and December 2001 386 patients with newly diagnosed MM were enrolled in a randomized, multicenter clinical trial of single transplantation (TX-1; Arm A) vs. double transplantation (TX-2; Arm B) of autologous peripheral blood stem cells (PBSC). In both arms of the study, treatment plan consisted of the following phases: conventional induction of remission with VAD×4 months; collection of PBSC with high-dose cyclophosphamide; first course of high-dose therapy (HDT) with melphalan at 200 mg/m² (MEL 200). Patients assigned to arm B received a second course of HDT with melphalan (120 mg/m²) and busulfan (12 mg/kg) after 3 to 6 months from TX-1. An analysis on an intent-to-treat basis was performed on 220 patients (of whom, 110 were randomized to TX-1 and 110 to TX-2) who were followed for a median of 3 years. The complete remission (CR)/near-CR rate was 53% in arm A and 72% in arm B. No statistically significant difference in overall survival (OS) was observed between the two groups (median, 62.5 months for TX-1 vs 74+ months for TX-2). Compared to TX-1, TX-2 conferred a significantly longer duration of remission (median, 23.5 months vs 39.5 months, respectively; p =.002) and resulted in extended event-free survival (EFS) (median, 31 months vs 21.5 months, respectively; p=0.002). The superiority of TX-2 over TX-1 was also confirmed by a landmark analysis of EFS performed at 5 months (p=.05) and by a multivariate Cox regression analysis that identified assignment to TX-2 as the most important and independent variable favorably affecting EFS (p=0.008). Patients who...
were more likely to benefit from Tx-2 in terms of significantly longer OS, EFS and time to progression (TTP) were those who did not initially respond to VAD and who failed to achieve CR following autologous transplantation. Within this latter subgroup, receiving TX-2 extended the 5-year projected from 40% (for TX-1 group) to 67.5% (p=0.04) and median EFS from 18.5 months (for TX-1 group) to 38.5 months (p=0.04). It is concluded that double autologous transplantation significantly improved both EFS and TTP in a series of 220 patients with previously untreated MM. Mature data derived from the final analysis of the trial must be awaited before definite conclusions can be given concerning the impact of double autologous transplantation on the ultimate outcome of MM.

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**MYELOMA AND PLASMA CELL DYSCRASIAS**

**C0055**

**CYCLIN D1 OVEREXPRESSION IS A FAVORABLE PROGNOSTIC VARIABLE FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH HIGH-DOSE CHEMOTHERAPY AND SINGLE OR DOUBLE PERIPHERAL BLOOD STEM CELL AUTOTRANSPLANT(S)**


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We analyzed the clinical and prognostic relevance of cyclin D1 overexpression, as evaluated by real-time RT-PCR, in a series of 74 previously untreated patients with multiple myeloma (MM) who were randomized to receive either a single or double autotransplants. In 46 of these patients conventional cytogenetics and eventually added FISH analysis were performed to investigate the presence of chromosome 11 and 13 abnormalities. We found in 98% of the patients analyzed a close correlation between elevated cyclin D1 mRNA levels and the presence of the t(11,14) or trisomy 11 (p<0.0001). In contrast, patients who overexpressed cyclin D1 and patients who did not were equally likely to have chromosome 13 monosomy (613) (41% vs. 38%, respectively; p=0.8). No difference in terms of age, gender, immunoglobulin isotype and concentration, stage at diagnosis, serum β2-microglobulin, bone marrow plasmacytosis, C-reactive protein and creatinine levels was recognized between patients who overexpressed cyclin D1 (group A; n=32) and patients who did not (group B; n=42). On an intention-to-treat basis, the complete remission rate and overall survival were similar for the two groups of patients. On the other hand, patients who overexpressed cyclin D1 had a significantly longer duration of remission in comparison with patients who did not (median, 41 vs.26 months, respectively; p=0.02). As a result, median event-free survival was longer in group A than in group B (median, 33 vs.24 months, respectively; p=0.055). Analysis of EFS in a subgroup of patients carrying 613 indicated that the presence of cyclin D1 overexpression was correlated with a better outcome in comparison with cyclin D1 negativity (median, 41 vs. 23 months, respectively). Similarly, the duration of remission for delta13 positive/cyclin D1 positive patients was longer than that observed in the delta13
positive/cyclin D1 negative subgroup (median, 41 vs. 26 months, respectively). It is concluded that cyclin D1 overexpression correlates strictly with 11q abnormalities and identifies a subset of MM patients who are more likely to have prolonged duration of remission and EFS following autotransplant(s), possibly counterbalancing the adverse prognostic relevance of 613.

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C0056

ANTI-IDIOTYPE VACCINATION OF MULTIPLE MYELOMA PATIENTS USING EX VIVO GENERATED DENDRITIC CELLS


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Thirteen multiple myeloma (MM) patients were treated with two courses of high-dose chemotherapy with peripheral blood stem cell support and then entered in a clinical study of anti-idiotype (Id) vaccination with dendritic cells (DC). DC were generated from positively selected circulating monocytes according to good manufacturing practice guidelines, in FCS-free medium in selected circulating monocytes according to good manufacturing practice guidelines, in FCS-free medium in presence of GM-CSF plus IL-4 followed by either TNF-α or a cocktail of IL-1β, IL-6, TNF-α, and prostaglandin-E2. CD14+ monocytes were enriched from 16.1±5.7% to 95.5±3.2% (recovery 67.9+/-15%, viability >97%). After cell culture, phenotypic analysis showed that 89.6±6.6% of the cells were DC: we obtained 2.89±167.9+/-15% of pre-loaded DC. The recovery of thawed, viable DC was 78±10%. Ten patients received a series of bi-monthly immunizations consisting of three subcutaneous and two intravenous injections of Id-keyhole limpet hemocyanin (KLH)-pulsed DC (5×10^6, 50×10^6 cells and 10×10^6, 50×10^6 cells, respectively). The patient-specific Id was used as whole protein in 4 patients whereas 6 additional patients had their DC charged with Id (VD)-derived HLA class I restricted peptides. The administration of Id-pulsed DC was well tolerated with no clinically significant side effects. So far, 6 patients have been fully evaluated for their immunological response to DC vaccination. Six of 6 patients developed a humoral and T-cell proliferative response to KLH. Moreover, 5/6 showed circulating IFN-γ-secreting T cells by Elispot. Conversely, none of the patients mounted a B-cell response to Id whereas 6/6 developed an Id-specific T-cell proliferative response and 4/6 IFN-γ-secreting T cells. Delayed-type hypersensitivity (DTH) tests showed 6/8 and 2/8 patients responsive to KLH and tumor Id, respectively. With a median follow up of 10 months, 7/10 patients have stable disease, 1 patient is in molecular CR and 2 patients progressed. In summary, positive selection of circulating CD14+ monocytes allows the generation of mature and functional DC suitable for clinical trials and cryopreservation does not affect the phenotype and function of pre-loaded DC.

Injections of cryopreserved Id-pulsed DC are safe and able for clinical trials and cryopreservation does not modify the phenotype or functional characteristics of pre-loaded DC. The cytokine cocktail induced a significantly higher percentage and yield of CD14+ cells than TNF-α alone, secretion of large amounts of IL-12, potent stimulatory activity on alloreactive and autologous T cells. Storage in liquid nitrogen did not modify the phenotype or functional characteristics of pre-loaded DC. The recovery of thawed, viable DC, was 78±10%. Ten patients received a series of bi-monthly immunizations consisting of three subcutaneous and two intravenous injections of Id-keyhole limpet hemocyanin (KLH)-pulsed DC (5×10^6, 50×10^6 cells and 10×10^6, 50×10^6 cells, respectively). The patient-specific Id was used as whole protein in 4 patients whereas 6 additional patients had their DC charged with Id (VD)-derived HLA class I restricted peptides. The administration of Id-pulsed DC was well tolerated with no clinically significant side effects. So far, 6 patients have been fully evaluated for their immunological response to DC vaccination. Six of 6 patients developed a humoral and T-cell proliferative response to KLH. Moreover, 5/6 showed circulating IFN-γ-secreting T cells by Elispot. Conversely, none of the patients mounted a B-cell response to Id whereas 6/6 developed an Id-specific T-cell proliferative response and 4/6 IFN-γ-secreting T cells. Delayed-type hypersensitivity (DTH) tests showed 6/8 and 2/8 patients responsive to KLH and tumor Id, respectively. With a median follow up of 10 months, 7/10 patients have stable disease, 1 patient is in molecular CR and 2 patients progressed. In summary, positive selection of circulating CD14+ monocytes allows the generation of mature and functional DC suitable for clinical trials and cryopreservation does not affect the phenotype and function of pre-loaded DC. Injections of cryopreserved Id-pulsed DC are safe and induce T-cell tumor-specific responses.
serum calcium 9.3 mg/dl (7-14.7). Conventional cytogenetic (or FISH) analysis was performed in 60 patients: 36 had a normal karyotype, 3 cases had del 13q, 1 case had del 11q, 3 others had a complex karyotype, and in the remaining 17 the assay was not informative. Following high-dose cyclophosphamide and granulocyte-colony stimulating factor, all patients mobilised enough peripheral hematopoietic stem cells (at least 6 millions CD34+/kg), which underwent negative selection in 41 cases (Barbuli et al., Br J Haematol, 2002;116:202). Results: Seventy-nine patients (86%) completed the program, and the other 13 received only one autotransplant for B virus hepatitis (n 6), bad performance status (n 1), cardiomyopathy (n 1), disease progression and death after the 1° autotransplant (n 3), severe neuropathy (n 15), and the other 13 received only one autotransplant. Results: Seventy-nine patients (86%) completed the program, and the other 13 received only one autotransplant for B virus hepatitis (n 6), bad performance status (n 1), cardiomyopathy (n 1), disease progression and death after the 1° autotransplant (n 3), severe neuropathy (n 15), and the other 13 received only one autotransplant.

Conclusions: TT-\(T^2\)=0.03). This chemotherapeutic oral protocol is efficacious and safe for treating patients with WM; moreover, results in overall survival are not worse than those obtained with more aggressive and expensive protocols based on new drugs.

CO058
A COMBINATION OF MELPHALAN, CYCLOPHOSPHAMIDE, AND PREDNISONE FOR TREATING PATIENTS WITH WALDENSTRÖM’S MACROGLOBULINEMIA
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Introduction. Waldenström’s macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by high levels of IgM immunoglobulin, lymphoplasmocytic bone marrow infiltration ± lymphadenopathy and splenomegaly. Standard therapy for patients with symptomatic WM is considered the oral administration of alkylating agents; however, reports about treatment are scanty, and due to the low prevalence of WM, most of the series are based on a low numbers of patients. We report here the results obtained in newly diagnosed WM patients using an oral chemotherapeutic regimen combining melphalan, cyclophosphamide and prednisone (MCP). Design and Methods: From July 1973 to April 2002, we observed 98 newly diagnosed consecutive WM patients. Of these, 26 were asymptomatic and did not require any treatment. The remaining 72 patients (46 males and 26 females) were treated because of the presence of anemia, hyperviscosity syndrome, hemorrhagic manifestations, significant cytopenias or extensive lymphadenopathy and/or hepatosplenomegaly. The laboratory and clinical characteristics of the treated patients were: median age 61 years (42-87 yrs), median monoclonal IgM 3.6 mg/dL (range:1.5-6.7 mg/dL), Median Hb levels 12g/dL (range: 7-16 g/dL). There were 70% of the patients with Hb levels <10 g/dL, 20% had hyperviscosity syndrome, 31% had splenomegaly and 10% had superficial adenopathy. Treatment consisted of melphalan (6 mg/m\(^2\)) + cyclophosphamide (125 mg/m\(^2\)) + prednisone (40 mg/m\(^2\)) administered orally from day 1 to 7 of each course, repeated every 4-6 weeks for a total of 12 courses. Maintenance treatment consisted of daily chlorambucil (3 mg/m\(^2\)) and prednisone (6 mg/m\(^2\)) until progression. Only patients who completed at least one course of treatment were considered evaluable. Responding and stable patients received the maintenance treatment until progression. Results: Among 72 treated patients, only one patient was lost to follow-up few days after initiation of therapy; therefore, a total of 71 patients were available for response to therapy. A response, defined as a reduction > 50% in the serum IgM initial levels, was achieved by 56/71 (80%) evaluable patients and 10 of these (18%) had a reduction > 75% in the serum IgM level. Moreover, additional 7 (9.8%) patients had a reduction < 50% but > 25% of the initial IgM levels with disappearance of all clinical symptoms and a reduction of lymphadenopathies and splenomegaly. The remaining 8 (10.2%) patients had disease progression, including one patient who developed a histologic transformation in non-Hodgkin aggressive lymphoma. After a follow up ranging from 1.2 yrs to 17.4 yrs (median 6 years), median event free survival and overall survival were 3.8 yrs and 6.6 yrs respectively with 14/71 patients still alive and 11 still responding. Therapy was well tolerated and acute toxicity was limited to transient nausea, vomiting and mild neutropenia. Conclusions: This chemotherapeutic oral protocol is efficacious and safe for treating patients with WM; moreover, results in overall survival are not worse than those obtained with more aggressive and expensive protocols based on new drugs.
**CO059**

**A PROSPECTIVE RANDOMIZED TRIAL OF INTERMEDIATE DOSE MELPHALAN (100 mg/m²) VS ORAL MELPHALAN/PREDNISONE**


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A clinical relationship between dose-intensity of melphalan and response rate has been demonstrated in multiple myeloma. Melphalan 200 mg/m² with autologous stem cell rescue is an effective treatment for myeloma patients who are younger than 65 years. It is uncertain whether melphalan 100 mg/m² (MEL100) can offer similar results in older patients. In a case-matched control analysis, we previously demonstrated that MEL100 was superior to MP in terms of response rate, event-free survival (EFS) and overall survival (OS). To definitively address this issue, a multicenter randomized study was performed. One-hundred and ninety-five myeloma patients, under the age of 70 years, entered the study between October 1997 and December 2000. Patients were randomly assigned at the time of diagnosis to receive either conventional oral melphalan and prednisone or 2 courses of MEL100 followed by stem cell support. Complete remission was 29% after MEL100 and 6% after MP (p<0.001). Partial remission was 49% after MEL100 and 37% after MP (p=0.61). No response were 21% after MEL100 and 57% after MP (p<0.001). Median event-free survival was 28.5 months in the MEL100 group and 16.4 months in the MP group (p<0.001). The probability of overall survival for 50 months was 72% in MEL100 group and 40% in MP group (p<0.01). Treatment related mortality was 4% after MEL100 and 1% after MP (p=0.11). In conclusion, MEL100 is an effective first line treatment for elderly myeloma patients. MEL100 is superior to MP in terms of complete remission rate, event-free survival and overall survival.

**CO060**

**COMBINED THALIDOMIDE-DEXAMETHASONE THERAPY FOR PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA**


In January 2002, we started a phase II multicenter study with combined thalidomide-dexamethasone as primary therapy for patients with newly diagnosed MM. By study design, thalidomide-dexamethasone were administered for 4 months in an attempt to reduce tumor cell mass before collection of autologous peripheral blood stem cells (PBSC) with high-dose cyclophosphamide (HD-CTX) and two subsequent transplantations of PBSC to support two sequential courses of melphalan at 200 mg/m². The starting dose of thalidomide was 100 mg/d, with a subsequent increase to 200 mg/d after 14 days; the monthly dose of dexamethasone was 40 mg/d on days 1 to 4, with courses repeated on days 9 to 12 and 17 to 20 on the first and third month of therapy. The first 50 patients who entered the study were evaluated for response, toxicity and collection of PBSC. Eighty-six percent of these patients were in advanced clinical stage and approximately 50% of them had high-risk MM, as identified by the presence of chromosome 13 abnormalities. On an intent-to-treat basis, the rate of at least or more than partial remission (PR) was 70%, including 4 who attained stringently defined complete remission and 7 additional patients with more than 90% decrease in their M protein concentration. Among responders, a decrease in serum M protein concentration by at least 50% above pretreatment levels was registered in 77% of the cases at the end of the first month of therapy. There was no apparent difference in the rate of at least or more than PR between patients with and without monosomy of chromosome 13 (65% vs 77%, respectively); however, the corresponding rates of progression for the two groups of patients were 22% vs. 4%. Side effects with thalidomide were mild in most of the patients and did not require dose reductions. Grade 3-4 toxicities included constipation (14%), fatigue (10%), neuropathy (6%) and skin rash (2%). In addition, deep vein thrombosis (DVT) was a troublesome adverse event, with an overall frequency of 18% (26% among the first 19 patients and 12% among the subsequent 31 who received fixed low-dose prophylactic warfarin (1.25 mg daily). Overall, 4 patients (8%) required thalidomide discontinuation due to toxicity. Following thalidomide-dexamethasone therapy, 3 patients (6%) failed to collect the minimum threshold dose of 2×10⁶/kg CD 34+ cells, while in the remaining patients the median number of collected CD 34+ cells was 7×10⁶ CD34+ cells/kg. It is concluded that combined thalidomide-dexamethasone has definite antitumor activity as primary therapy for MM. Based on the ease of oral administration, relatively mild extramedullary toxicity and apparent lack of PBSC injury, thalidomide-dexamethasone deserves further investigation as an alternative to VAD in patients who are candidates to subsequent autologous transplantation.

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LYMPHOPROLIFERATIVE SYNDROMES I

PO001
OUTCOME OF ADVANCED CLL PATIENTS RELAPSED AFTER AN AUTOLOGOUS STEM CELL TRANSPLANTATION
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Between 1995 and 2002, 30 chronic lymphocytic leukemia (CLL) patients (pts), median age 46 years (range 22-59), with advanced disease (Binet stages B or C), who achieved clinical complete remission (CR) (24 pts) or partial remission (PR) (6 pts) after fludarabine (FLU), were submitted to HD-Cyclophosphamide to collect peripheral blood stem cells (PBSC) and were autografted at our Institution. Four of 30 pts failed to collect PBSC and underwent a marrow collection and reinfusion. The BEAM (BCNU, etoposide, Ara-c, melphalan) conditioning regimen was utilized in most pts (24). At the time of transplant, all pts were in clinical CR: 18 pts after one line of therapy, 5 after two lines and 7 after three or more lines, with a median interval from diagnosis to transplant of 41 months (range 8-131). The overall survival probability is 0.62, projected to 95 months from transplantation. Eighteen of the 30 pts (60%) showed a clinical relapse after a median time from transplant of 31.5 months (range 2-79). Neither the number of lines of therapy before transplant (1 or 2 lines) nor the interval from diagnosis to transplant (< 36 or ≥ 36 months) significantly influenced progression-free survival probability. Two of 18 pts have not required treatment after 25 and 5 months from relapse, while 16 have undergone treatment with a median interval from clinical relapse to treatment of 3.5 months (range 1-38). Two of 16 patients were refractory after two lines of therapy (chlorambucil (CB), FLU plus cyclophosphamide (CY): 1 pt; CB, vincristine: 1 pt). Thirteen of the 16 patients (81.2%) achieved a clinical partial response: 11 (84.6%) after a first line of therapy (CB: 6 pts; Rituximab alone: 3 pts; FLU plus CY: 1 pt; PVABEC regimen: 1 pt) and 2 after two lines of therapy (CB, FAND plus Campath: 1 pt; Rituximab, FAND: 1 pt). One out of 16 patients achieved a clinical complete remission after a first line of therapy (CB plus FLU plus CY plus Rituximab). The median response duration was 10 months (range 1-36). Overall, 14/18 relapsed patients are alive with a median follow-up of 29.5 months after relapse (range 5-50), of 68 months after transplant (range 15-95) and of 99.5 months after diagnosis (range 43-142), while 4 patients have died because of disease progression after 46, 36, 32 and 31 months from relapse, 70, 67, 47 and 71 months from transplant, and 112, 134, 149 and 105 months from diagnosis, respectively. Our experience suggests that the majority of CLL patients relapsing after an autograft have a chance of achieving a new response with chemotherapy, with a median survival of 46 months from relapse and of 149 months from diagnosis.

PO002
CLINICAL SIGNIFICANCE OF SOLUBLE APOPTOTIC MOLECULES IN B-CHRONIC LYMPHOCYTIC LEUKEMIA
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B-chronic lymphocytic leukemia (B-CLL) symptomatic patients are often refractory to conventional treatment and this problem seems to be in part related to the fact that B-CLL cells are resistant to chemotherapeutic-induced apoptosis. However, when cultured in vitro, spontaneous apoptosis occurs, suggesting the existence in vivo of survival-promoting factors, among them increased levels of soluble apoptotic molecules, such as Fas and annexin V, that inhibit the receptorial apoptotic pathway, and Bcl-2, an anti-apoptotic mitochondrial protein. Soluble forms of CD95 and CD95 ligand in human serum are able to block Fas/Fas ligand interaction and thus prevent apoptosis. Also, IL-18, an immunoregulatory cytokine, downregulates Fas-mediated apoptosis increasing the protein expression of p53 and Fas ligand and high levels were found in acute myeloid leukemias. In order to define the prognostic impact of these apoptotic molecules on the clinical outcome of B-CLL, we investigated 183 patients, median age 65 years, 98 males and 85 females. Soluble CD95 (sCD95), soluble Annexin V (sAnnexin V), and IL-18 were assayed on plasma of peripheral blood samples by immunoenzymatic methods. Bcl-2 protein (sBcl-2) was determined on leukocyte lysate samples by an ELISA procedure. The thresholds of positivity were set at >7.5 ng/mL for sCD95, >2 ng/mL for sAnnexin V, >500 pg/mL for IL-18 and >400 U/mL for sBcl-2. With regard to patients characteristics, 51 had low Rai stage, 126 intermediate stage and 6 high stage.
Within the intermediate/high Rai stages, there were 71/79 IL-18+ patients (p=0.0005), 38/41 sAnnexin V+ cases (p=0.00001), 47/55 sCD95+ pts (p=0.0006) and 41/43 Bcl-2+ patients (p=0.0003). β2-microglobulin >2.2 mg/mL was significantly associated with IL-18 >500 pg/mL (54/79; p=0.00001), sCD95 >7.5 ng/mL (41/54; p=0.00001), sBcl-2 > 400 U/mL (32/42; p=0.0001) and sAnnexin V >2 ng/mL (29/41; p=0.004).

Furthermore, the presence of three or more intrathoracic/abdominal lymphadenopathies (>3 cm in diameter) and/or splenomegaly was significantly correlated with higher IL-18 (39/79; p=0.01), higher sCD95 (32/55; p=0.0001), higher sAnnexin V (24/41; p=0.008) and higher sBcl-2 (23/43; p=0.04). Within a group of 54 patients treated with fludarabine, a higher complete remission (CR) rate was found only in sAnnexin V negative patients (87% vs 13%; p=0.001). Moreover, shorter progression-free survival (PFS) and overall survival (OS) were observed in IL-18+ patients (25% vs 67% at 9 years; p=0.002 and 41% vs 95% at 11 years; p=0.0005), in sCD95+ patients (28% vs 54% at 9 years; p=0.01 and 46% vs 64% at 11 years; P=0.01), in sBcl-2+ patients (17% vs 76% at 9 years; p=0.00008 and 23% vs 71% at 11 years; p=0.00007) and in sAnnexin V+ patients (13% vs 56% at 9 years; p=0.0002 and 35% vs 78% at 11 years; p=0.01). In multivariate analysis, sBcl-2 was confirmed to be an independent prognostic factor with regard to PFS (p=0.01) together with CD38 expression (p=0.005). Therefore, these soluble apoptotic proteins, mainly sBcl-2, represent significant biological parameters to enucleate high risk subsets. The considerable impact of apoptotic pathways on the clinical course of B-CLL patients should encourage the experimental use of apoptosis-targeted drugs in combination with chemotherapy in order to increase the response rate and to prolong time to progression and survival.

**P0003**

**GENE EXPRESSION ANALYSIS AND IMMUNOGLOBULIN MUTATIONAL STATUS IN CHRONIC LYMPHOCYTIC LEUKEMIA**


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B-cell chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disorder characterized by the accumulation of clonal, small B-lymphocytes co-expressing B-cell-associated antigens as well as the CD5 molecule and surface immunoglobulins, in general IgM and IgD. The clinical course of CLL patients is quite variable, with many patients surviving for prolonged period without therapy, whereas other succumb rapidly despite aggressive treatment. Despite having several phenotype characteristics of naïve B1a cells, CLL have been shown to have somatically mutated immunoglobulin variable region genes in more than 50% of cases. According to this fact, CLL patients can be divided into two subgroups with different clinical behaviour and survival. Here we report the expression levels analysis of specific genes known to be differentially expressed in CLL subsets as well as of different genes involved in lymphocytes differentiation and in the germinal centre reaction according to the immunoglobulin mutational status. The status of Ig genes was defined analysing the nucleotide sequence of expressed rearranged immunoglobulin heavy chain (IgH) genes obtained by direct sequencing of RT-PCR products of 122 well characterized B-CLL cases. Somatic mutations were found in 70 cases (58%), including 20 cases (27.8%) with a low rate of mutations (2 to 5% base substitutions)(M-CLL). The remaining 52 cases were defined unmutated (UM-CLL), as defined by the presence of a rate of base substitution lower than 2%. Mutational status well correlated with overall survival showing a median survival of 16.9 years of the mutated group and of 6.3 years of the mutated one (p: 0.0052). Moreover, unmaturated patients seem to have a more aggressive disease requiring treatment in the great majority of cases (73.2%) that was necessary in only the 34.6% of the M-CLL (p:0.0001).

Large scale gene expression analysis was assessed by microarray technology in order to identify specific expression gene profile specifically related to mutated or unmutated immunoglobulin status. Total RNA extracted from 8 cases (4 M-CLL and 4 UM-CLL) was converted into labelled cRNA and hybridized to U95A Affymetrix Gene Chips representative of about 12,000 genes. Gene profiling was analysed by supervised clustering in order to compare the two CLL cell populations defined for the presence of Ig somatic mutations. Although the low number of cases examined, the analysis showed the presence of a small number of genes differentially expressed among the two CLL groups. Expression of different genes were analysed by Real-TimePCR on 50 CLL cases indicating a statistical correlation between Ig mutational status and a higher expression in UM-CLL of BCL6, CD38 and ZAP-70 genes. Moreover, very different expression levels in different samples were found also for the FAS and A-MYB genes that do not correlate with the IgH status. The same samples were found also for the FAS and A-MYB genes that do not correlate with the IgH status. The same examination showed a statistical correlation between Ig mutational status and a higher expression in UM-CLL of BCL6, CD38 and ZAP-70 genes. Moreover, very different expression levels in different samples were found also for the FAS and A-MYB genes that do not correlate with the IgH status. The same samples were found also for the FAS and A-MYB genes that do not correlate with the IgH status. The same examination showed a statistical correlation between Ig mutational status and a higher expression in UM-CLL of BCL6, CD38 and ZAP-70 genes. Moreover, very different expression levels in different samples were found also for the FAS and A-MYB genes that do not correlate with the IgH status. The same samples were found also for the FAS and A-MYB genes that do not correlate with the IgH status.

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The mutational status of IgVH genes is, along with CD38 expression, a prognostic marker in early stages of B-cell chronic lymphocytic leukemia (B-CLL). Configuration of IgVH genes with more than 2% mismatch (mutated, M, B-CLL) correlates with longer survival. In an unselected series of 69 B-CLLs (46 male and 23 females; median age 64.0 years, range 32-97), we analyzed the mutational status of IgVH genes (67/69) and the expression of CD38 (64/69). By using the 2% mutation cut-off, 44.8% (30/67) of cases were M-B-CLL, while expression of CD38 above 30% of positive cells was found in 45.3% (29/64) of cases. A retrospective comparison with survivals was possible in 58 (CD38) and 61 (IgVH mutations) cases. While survival curves demonstrated the significance of CD38low as a marker of good prognosis (p = 0.041), we were not able to confirm the prognostic value of IgVH mutational status by utilizing the 2% mutation cut-off (p = 0.275). Since M-B-CLL may derive as a transformation of post-germinal center (GC) B cells as the result of a positive selection operated by the antigen, we investigated whether the mutational status was or not consistent with that of B cells which underwent antigen-driven selection. This was accomplished by applying statistical methods evaluating a significant skewing of replacement mutations from framework (FR) to complementarity-determining (CDR) regions. According to this approach, 27/67 cases displaying a significant excess of R mutations in CDRs and/or scarcity in FRs (significantly mutated, sM, B-CLL). These patients had longer survivals both if compared with all the other cases, i.e. UM (< 2% mutations) and not significantly mutated (nsM, > 2% mutations) B-CLLs (p=0.010), as well as with the groups of nsM (p=0.022) and UM (p=0.042) B-CLLs alone. The superior survival of sM-B-CLL patients held true, although at borderline significance (p=0.050) also when the good prognosis group of CD38low B-CLLs was separately analyzed. These data give, for the first time, formal proof of a better prognosis for those B-CLL cases which have arisen from post-GC B cells and suggests a certain caution in a wide application of the cut-off of 2% somatic mutations as a prognostic determinant, without demonstration of antigen-driven selection.

PO005
LONG-TERM FOLLOW-UP OF HAIRY CELL LEUKEMIA PATIENTS FRONT-LINE TREATED WITH 2-CHLORODEOXYADENOSINE
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The management of patients with hairy cell leukemia (HCL) has evolved significantly over the past two decades. In fact, both 2’-deoxycoformycin (DCF) and 2-chlorodeoxyadenosine (2-CdA) induce complete response (CR) in the majority of the patients with HCL. However, fewer data exist on the long-term follow-up of patients who have undergone the characteristically brief exposure to 2-CdA therapy. Thus, it is important to evaluate such long-term outcome data in order to better understand the efficacy of this agent in the management of HCL. We reviewed the long-term follow-up data of 37 untreated HCL patients treated with a single course of 2-CdA administered as a single continuous IV infusion for 7 days at the dose of 0.1 mg/kg/day or at a dose of 0.15 mg/kg/2 hours infusion once a week for 6 courses or at a dose of 0.15 mg/kg/day 2 hours infusion for 5 consecutive days in our institute from January 1991 and February 2001. Of 37 patients, 29 (78%) achieved a CR and 6 (16%) a partial response (PR), with an overall response rate of 94%. After a median follow-up of 90 months (range: 20-168), there have been 8 (27%) relapses. All these relapses occurred between 42 and 128 months. All relapsed patients were re-treated with 2-CdA at the dose of 0.15 mg/kg/day for 5 days in a 2-hour infusion, and 75% and 25% then obtained CR or PR, respectively. The median duration of this second response was 50 months (range: 36-96). All but one of these patients are still maintaining the second response to 2-CdA. The 14-year overall and the relapse-free survivals are 96% and 52%, respectively. In HCL patients a single dose of 2-CdA induces a long-term CR with a 14-year survival > 90%. Over 50% of patients tend to appear clinically cured by this procedure, but the lack of a long-term plateau in the relapse-free survival curve still warrants caution on this point.
INCIDENCE OF SECOND NEOPLASIA IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: RETROSPECTIVE ANALYSIS ON 165 PATIENTS TREATED WITH CHLORAMBUCIL MAINTENANCE CHEMOTHERAPY

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The incidence of second neoplasia (SN), retrospectively analyzed on 642 B-cell Chronic Lymphocytic Leukemia (B-CLL) patients consecutively observed in our institution, resulted 14.8% (95/642); in particular 79 cases (12.3%) developed an epithelial cancer and 15 (2.5%) underwent transformation into aggressive non Hodgkin Lymphoma (Richter Syndrome). In the remaining case, a diagnosis of Thrombocytopenia was performed some years before B-CLL detection. In this study we investigated the impact of prolonged maintenance therapy with Chlorambucil, given twice a week, with a 5-15 mg/day schedule, in 165 responding patients belonging to this series. After excluding 5 cases who developed SN before any treatment start, 160 patients were accounted, 93 males and 67 females, median age 66 years (range 43-91) and with the following stage distribution at diagnosis: 94 in stage A according to Binet, 45 B, 14 C; 32 stage 0 according to Rai, 103 stage I-II, and 18 stage III-IV. The median maintenance duration was 36 months (range 7-120) and after a follow-up of 78.6 months (range 7-303), 31 patients (19.3%) underwent SN. Gastrointestinal tract (32.2%), lung (22.5%), skin (19.3%) and prostate (3.2%) were the most frequently involved sites. Richter syndrome occurred in the remaining 22.5% of cases. No case of acute leukemia or myelodysplastic syndrome has been recorded. The majority of hairy cell leukemias (HCL) have been shown to derive from a cell which has undergone mutation in VH genes with a low level of intraclonal heterogeneity, and expressing multiple isotype transcripts in single hairy cells. This suggested arrest at a stage of isotype switch where RNA processing events precede deletional recombination. In the present study, we have investigated the stage of arrest in HCL further, by correlating their VH gene status with Ig isotype, AID, with an induction therapy without maintenance. Further analyses are actually in progress with the aim to compare this subset of patients to cases undergoing SN without receiving any treatment and to cases treated with an induction therapy without maintenance.

UNMUTATED AND MUTATED VH GENES REVEAL HETEROGENEITY IN HAIRY CELL LEUKEMIA, WITH BOTH SUBSETS EXPRESSING MULTIPLE ISOTYPES AND AID BUT LACKING CD27 AND CD38

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Analysis of immunoglobulin (Ig) variable (V) region genes provides insight into the clonal origins of B-cell tumors. Normally, naive IgM/D-positive B-cells have not initiated somatic hypermutation (SHM), generally localised to the germinal center (GC). Following antigen encounter in a mature B-cell, SHM and isotype switch are initiated in a GC reaction. There is a critical role for activation-induced-cytidine-deaminase (AID) in both processes. Post-GC B-cells have generally completed SHM and differentiate either into CD27+ memory B-cells or into CD38+ve plasmablasts. CD38 is also a marker of early events in B-cells during initial stages of the GC reaction, and reappears on Ig secreting plasma cells. The majority of hairy cell leukemias (HCL) have been shown to derive from a cell which has undergone mutation in VH genes with a low level of intraclonal heterogeneity, and expressing multiple isotype transcripts in single hairy cells. This suggested arrest at a stage of isotype switch where RNA processing events precede deletional recombination. In the present study, we have investigated the stage of arrest in HCL further, by correlating their VH gene status with Ig isotype, AID, CD27 and CD38 expression. Eleven of 13 cases revealed mutated VH genes (range 93.5-98.6%) with a low level of intraclonal heterogeneity confirmed (10/II). Heterogeneity also established a mutated status in cases displaying 98% homology. Notably, 2/13 HCL displayed germline VH genes (100% homology), and both cases were V3-30-derived. Both mutated (10/II) and unmutated cases (2/2) co-expressed pre (IgM and/or D) and post-switch (IgG and/or A) isotypes on the hairy cells. AID transcript was expressed in 10/11 HCL, and a loss of exon 4 AID variant was additionally identified in 8/10 cases. In 9/10 AID+ cases, switching was apparent, but AID did not correlate with SHM in 2 cases (1 mutat-
ed HCL AID-, 1 unmutated HCL AID+). Of note, CD27 and CD38 expression was absent in all cases. Our analysis demonstrates that HCL can be now be divided into a VH gene mutated and a novel unmutated subset. Both subsets can isotype switch, and for unmutated HCL, this raises the question of at which site this may be occurring. AID tends to associate with IS, but not always with SHM, suggesting a potential dissociation. Both subsets of HCL lack CD27 and CD38, suggesting either an aberrant pattern of expression, or neoplastic arrest prior to acquisition of these markers in the mutated subset. VH genes now reveal heterogeneity of HCL, with patterns of AID, CD27 and CD38 expression being common.

PO008
CLINICAL AND PROGNOSTIC FEATURES OF CD5 NEGATIVE MATURE B CELL LEUKEMIAS: A RETROSPECTIVE STUDY ON 139 PATIENTS PERFORMED BY INTERGRUPPO ITALIANO LINFOMI
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Introduction: CD5 (and CD10) negative mature B-cell leukemias (M BCL) are a group of chronic disorders that are considered the leukemic phase of indolent lymphomas like lymphoplasmacytic lymphoma and marginal lymphoma origin (excluding the well defined forms of hairy cell and prolymphocytic leukemia). Those lymphomas are characterized by an indolent course but have never been extensively studied so far. The IIL has retrospectively collected data on 139 patients with CD5 negative M BCL and performed a study with the aim of defining their clinical behaviour and to evaluate prognostic features. Patients and methods: patients included into the study patients should have a peripheral lymphocyte count above 5x10⁹/L at presentation with an immunocytomorphologic diagnosis of CD5 negative mature B-cell leukemia. So far 118 patients are evaluable, 18 are pending because of missing or unconfirmed data and 3 were excluded due to date of diagnosis. Results. The median age of the 118 patients was 68 years (range 40-88), and male/female ratio was 0.96. Sixty-six percent of the patients had Binet stage A, 14% stage B and 20% stage C; splenomegaly was present in 64% of the cases; 10% of patients presented with poor performance status (ECOG PS > 1) and 17% had B symptoms at diagnosis. As far as laboratory data are concerned median lymphocyte count was 10.4x10⁹/L (range 5.1-111); anemia (Hb <11 g/dL) was present in 25%, thrombocytopenia (plt <100x10³/L) in 14%, ESR >30mm in 34%, elevated β2microglobulin in 76%, abnormal LDH in 28%. A serum monoclonal component was detected in 35 (30%) patients being of G, A and M class in 17, 2 and 16 patient respectively. Treatment varied among institutions and time of diagnosis: 79 patients were initially addressed to a watch and wait policy; only 26 of those required treatment during follow up, defining a 3 years freedom from treatment survival of 65%. Chemotherapy regimens were as follows: single agent 66%, CVP-like 3%, CHOP-like 28%, Flu darabine 3%. After a median follow-up of 36 months (range 1-160 months), 3 and 5-years OS rates were 88% and 81% respectively. In univariate analysis only the presence of advanced age (>60 years), elevated LDH and of splenomegaly correlated with a poorer survival (p=0.009, p=0.0001 and p=0.04 respectively). International Prognostic Index model was able to identify different groups of patients (23, 47, 26 and 4% of patients with an IPI score of 1, 2, 3 and 4 respectively), with significant different OS rates (p=0.0001) varying from 100% 5 year-OS for patients with up to one adverse parameter to 82% for those with 2, to 58% for those with 3 or more adverse features. Conclusions: The preliminary analysis on 118 cases showed that CD5 negative M BCL are leukemias characterized by an indolent clinical course with variable presenting features. Further analysis will allow the identification of patients with M BCL characterized by poorer survival.

PO009
B-CHRONIC LYMPHOCYTIC LEUKEMIA CELLS EXERT IN VITRO CYTOTOXICITY MEDIATED BY TUMOR NECROSIS FACTOR α
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Tumor necrosis factor α (TNFα) is constitutively produced by B-chronic lymphocytic leukemia (B-CLL) cells and acts as an autocrine factor for their growth and survival. However, there is little information on the possible cytotoxic effects which TNFα released by B-CLL...
cells can exert on different cell types. In this study, we investigated whether B-CLL cells in vitro may be cytotoxic against TNF-α-sensitive hematopoietic cell lines of different origin by TNFα production. Furthermore, since TNFα production is modulated by several cytokines, we examined the effect of interleukin (IL)-2 and IL-12 on TNF-α release by B-CLL cells and B-CLL cell cytotoxicity. We chose these cytokines on the basis of other studies demonstrating that T cells and dendritic cells from patients with B-CLL have an increased ability, compared to cells from normal subjects, for producing in vitro IL-2 and IL-12, respectively, both spontaneously and after activation. B-CLL cells were purified from blood of 12 patients. Cytotoxicity of B-CLL cells and supernatants of B-CLL culture against TNFα-sensitive Jurkat, U937 and K562, and TNFα-resistant Raji cells was measured by an 18-hour 51chromium release assay. TNFα in the supernatants of B-CLL cultures was quantified by enzyme-linked immunosorbent assay (ELISA). TNFα gene expression was analyzed by Northern blotting. In 8 of 12 patient samples examined (66.6%), B-CLL cells, at an E:T ratio of 100:1, in vitro killed Jurkat, U937 and K562 cells (21.4±1.1%, 13.3±3.9%, 6.5±2.8% cytotoxicity, respectively), but not Raji cells. The following results support the involvement of TNF-α in B-CLL cell cytotoxicity: 1) it was strongly reduced by anti-TNF-α neutralizing antibodies (Abs), 2) TNF-α was detected in the supernatants of cytotoxic B-CLL cells, 3) these supernatants were cytotoxic against Jurkat, U937 and K562 but not Raji cells and this cytotoxicity was completely neutralized by anti-TNFα Abs. When B-CLL cells were stimulated for 24 hours with IL-2 (50 U/mL) and IL-12 (4 ng/mL) alone, release of TNFα was unchanged compared to unstimulated cultures, but when cytokines were added in combination, they induced a strong increase of TNFα release and mRNA expression. B-CLL cells stimulated with IL-2 plus IL-12 also exerted an increased cytotoxicity, which was related to the enhanced TNFα production, because anti-TNFα Abs strongly reversed this effect. In conclusion, our data show that B-CLL cells can kill in vitro TNFα-sensitive cell lines via TNF-α production and that this cytotoxicity is increased by IL-2 plus IL-12. These results raise the question of whether TNFα released by B-CLL cells can exert a cytotoxic activity in vivo.

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LOW DOSE SUBCUTANEOUS CAMPATH 1-H (C1H) THERAPY IS SAFE AND EFFECTIVE IN IN RELAPSING/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA
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Treatment of CLL with standard regimen C1H (30 mg x 3/w i.v. for 12 wks) can be associated with acute toxicity due to sudden cytokine release and to infections (mainly CMV reactivation). It has been recently shown that subcutaneous administration of C1H reduce immediate reactions and that a low dose schedule (10 mg x 3/w) for up to 18 weeks is as effective as standard dose. For these reasons we treated 8 patients affected by advanced CLL (M/F 7/1; median age 65 years, range 51-73; IWCLL clinical stage: IV in 4 cases, IIIb in 3, IIb in 1; median WBC count 58 x 10⁹/m³, range 19.7-122.9) with low dose subcutaneous C1H. To further reduce cutaneous reactions 5 mg hydrocortisone were injected together with C1H. Five patients had previously received 2 lines of chemotherapy and 3 patients received C1H as second line therapy because of previous occurrence of autoimmune hemolytic anemia, precluding further use of purine analogues. After a median treatment period of 5 weeks (range 2-12) these are our results: Safety: all patients are alive; no systemic adverse reaction and only 2 local transitory erythemas were observed; a cutaneous infection sustained by Pseudomonas aeruginosa (ecthyma gangrenosum) developed in one patient, previously treated with polychemotherapy including alkylating agents, fludarabine and rituximab. Despite patients’ recent clinical history was positive for CMV p65 antigenemia (1 pt), autoimmune hemolytic anemia (3 pts), infectious pneumonia (1 pt) and HBV positive viral hepatitis (1 pt), only one bacterial pneumonia was recorded and no other infections were observed, neither CMV reactivation; no autoimmune cytopenias were recorded. Efficacy: one of the patients showed refractoriness to therapy, one obtained CR according to the NCI criteria after 8 weeks of treatment and is now in a maintenance regimen with 2 monthly dose of C1H (10 mg); 5 patients obtained PR and are still on treatment; one patient is not evaluable (too early). Despite the small number of enrolled patients up to now, the low toxicity profile and the ease of administration are encouraging. The observed overall response rate of nearly 85%, before the completion of planned treatment, in this subset of advanced CLL is extremely interesting and deserve further investigation.

FAND REGIMEN PLUS RITUXIMAB: AN EFFECTIVE SALVAGE TREATMENT IN YOUNG PATIENTS WITH FLUDARABINE-RESISTANT B CELL CHRONIC LYMPHOCYTIC LEUKEMIA
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Patients with fludarabine-resistant or early relapsed B cell chronic lymphocytic leukemia (CLL) have few effective therapeutic options and generally a poor prognosis. Despite the remarkable advances in disease management allowed by the development of new and more effective salvage treatments, the outcome of this subset of patients is generally considered disappointing. Encouraging results from an intensive polychemotherapy regimen including fludarabine, ara-C, mitoxantrone and dexamethasone (FAND), have recently been reported. We report the following cases to bring attention to the possibility of sustained durable remission with anti-CD20 monoclonal antibody (rituximab) in patients with active and fludarabine-resistant disease who have obtained a good response after treatment with the FAND regimen. The series included three patients, all male, with a median age of 54 (48-59) years. Of these, two were in Rai stage III and one in stage IV disease. The FAND regimen included fludarabine (25 mg/m² i.v. days 1-3), ara-C (1 g/m² i.v. days 1-2), mitoxantrone 10 mg/m², i.v. day 1 and dexamethasone (20 mg i.v. days 1-3). Granulocyte colony-stimulating factor (G-CSF) was given on day 5 until to recovery. Two patients, presenting disease-related and therapy-induced anemia respectively, received epoetin alfa. Each FAND course was administered monthly and four courses were given on outpatient basis. All patients received the standard prophylactic measures for infectious complications. One month after the completion of salvage treatment, the patients received rituximab, 375 mg/m² once weekly for 4 consecutive weeks as consolidation therapy. Patients with objective response or stable disease after the rituximab therapy received scheduled maintenance courses of the same agent at 6-month intervals in an attempt to prolong the duration of remission and to delay the progression, according to the previously suggested role of rituximab in the long-term management of an other CD20 expressing lymphoid malignancy, the follicular non-Hodgkin’s lymphoma. According to NCI criteria, two patients achieved a complete remission (CR) and one a partial response (PR), given the persistence of a large spleen that was removed, presenting only minimal residual disease in the bone marrow after splenectomy. Hematologic toxicity included grade III-IV anemia, neutropenia and thrombocytopenia, that were recorded in 2/12 (16.6%), 12/12 (100%) and 6/12 (50%) cycles respectively. Grade II mucositis occurred in all courses. No patients required admission or transfusions of blood-derived
Patients with fludarabine-resistant B-Cell chronic lymphocytic leukemia (CLL) have few effective therapeutic options and alternative approaches are required in this setting to obtain long-term disease control. An intensive polychemotherapy regimen, including fludarabine, ara-C, mitoxantrone and dexamethasone (FAND), has shown highly effective and promising results (Mauro et al., Haematologica 2002). Recently, several reports have been published claiming the efficacy of anti-CD20 monoclonal antibody (rituximab) in the treatment of CLL, suggesting that this agent has activity against this disease as single agent or in association with fludarabine. We report our experience on a combined chemo-immunotherapy in three cases, focusing attention on the possibility of sustained durable remission with rituximab in patients with active and fludarabine-resistant disease, who have obtained a good response after salvage treatment with the FAND regimen. The three patients were male, with median age of 54 (48-59) years, 2 were fludarabine-resistant and 1 progressed within three months from the completion of the last fludarabine course. At the evaluation for FAND regimen, out of these, two were in Rai stage III and one in stage IV disease. They had no histocompatible sibling. All patients were properly informed and gave written consent. Patients received monthly courses including fludarabine (25 mg/m² i.v. days 1-3), ara-C (1 g/m² i.v. days 1-2), mitoxantrone 10 mg/m², i.v. day 1) and dexamethasone (20 mg i.v. days 1-3). Four courses were given on outpatient basis. Granulocyte colony-stimulating factor was given on day 5 until to recovery. One month after the completion of salvage treatment, the patients received the rituximab, 375 mg/m² once weekly for 4 consecutive weeks as consolidation therapy. Patients presented an almost stable response after rituximab and received another 4 maintenance courses, at 6-month intervals, in an attempt to prolong the remission and to delay progression, according to the previously suggested role of this agent the long-term management of another CD20 expressing lymphoid malignancy. Minimal residual disease (MRD) was monitored by immunopheno-typing and, in patients with < 10% bone marrow (BM) lymphocytes, by molecular analysis. According to NCI...
...criteria, the FAND regimen, achieved complete remission (CR) in two patients and a partial response (PR) in the third; last had persistence of a large spleen that was removed surgically. Hematologic toxicity included grade III-IV anemia, neutropenia and thrombocytopenia, which were recorded in 2/12 (16.6%), 12/12 (100%) and 6/12 (50%) cycles respectively. Grade II mucositis occurred in all courses. No patients required admission or transfusions. All patients had a residual CLL population in the BM and one also in the peripheral blood (PB), detectable by immunophenotyping, after completion of the FAND regimen. The treatment with rituximab was conducted according to standard dosage and schedule and the patient did not experience any severe side effect. Two patients completed the treatment while the third received 3 out of the four-planned courses. Response duration and overall survival lasted 20,26,30 and 28,34,36 months. The patient who previously underwent splenectomy, on the last assessment after the completion of the fourth rituximab course, achieved molecular remission. Another had no CD20/CD5 lymphoid cells in the BM and in PB after the third course of rituximab, molecular analysis is underway. In the remaining patient, although presenting no clinical signs of disease, 30% CD20/CD5 lymphocytes were detectable in BM by immunophenotyping and no improvement in MDR was seen after three rituximab course compared to evaluation after the FAND regimen. In spite of the remarkable improvements in recent years in CLL management, the therapeutic approach in young fludarabine-failed patients remains a clinical concern. We have presented here the favorable and long-standing response obtained with the FAND regimen followed by rituximab in three CLL patients who had been previously treated with fludarabine. Anti-CD20 was given as maintenance in repeated cycles in an attempt to prolong response duration. Second-line regimens in fludarabine-resistant CLL patients usually result in responses of limited duration, with a median time to progression of approximately 2 years. In this context, different strategies for consolidation of response have been proposed (mainly, stem-cell transplantation and treatment with Campath-1H). The duration of ongoing responses, ranging from 22 to 32 months, supports a favorable effect of maintenance with rituximab, shown by the improvement of the response achieved with FAND by clearance of residual CLL population in two out the three patients, one of these with a molecular remission. In spite of the few cases treated, the high efficacy of the FAND regimen in three fludarabine-resistant patients and the durable responses maintained by rituximab, lead us to hypothesize this strategy to prolong the time to progression.

Control of cell growth and differentiation in B-cell malignancies may be regulated by autocrine production of cytokines. In chronic lymphocytic leukemia (CLL) certain cytokines secreted by neoplastic B cells might prolong their survival, while others could limit the expansion of the neoplastic B cell clone. We recently reported that CD40 triggering upregulates TNF receptor I and II (TNFRI/II) on CLL B cells and that increased levels of TNF-α and IFN-γ, produced by CD40-activated cells, contribute to sensitize CLL B cells to fludarabine-induced apoptosis. Caspases-1 and -6 are the principal mediators of fludarabine- or dexamethasone-induced apoptosis in resting CLL B cells, but the same caspases (1 and 6) appear no longer involved after CD40-activation. Within a group of 10 CLL patients studied, we further observed that caspase-1 (ICE) inhibitor (YVAD-FMK), instead of inhibiting apoptosis, synergizes with fludarabine, and to a further extent with dexamethasone, in apoptosis induction of CD40-activated cells. Since caspase-1 is capable of mediating apoptosis but also of processing interleukin-1 β (IL-1β) precursor into the active form, we investigated IL-1 β release in resting and in activated cells. We could demonstrate that the levels of IL-1 β secreted were significantly higher in CD40-activated cells (mean±SD: 34 ±206 pg/mL) than in cells at resting conditions (39±93 pg/mL; p=0.006, student t test). Altogether these findings indicate a crucial role of caspase-1 activation and IL-1β secretion in counteracting fludarabine- and dexamethasone-induced apoptosis in CLL B cells after CD40 triggering. In agreement with this suggestion the addition of caspase-1 inhibitor, which neutralizes processing of pro-IL-1β into the mature IL-1β form, strongly amplifies apoptosis induced with the two drugs. These data further highlight as an altered profile of cytokines, secreted by activated neoplastic B cells, may condition their final response to pharmacological treatment.
**PO015**

MODULATION OF CD80, CD86, AND CD95 ON THE SURFACE FROM CLL PATIENTS BY PREACTIVATED AUTOLOGOUS T LYMPHOCYTES

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Profound immune dysfunction is a constant feature in B-cell chronic lymphocytic leukemia (B-CLL) patients. Immunological abnormalities include severe hypogammaglobulinemia, impaired immunoglobulin class switching, and diminished germinal center formation. This state of immune suppression renders B-CLL patients highly susceptible to infections, which contribute greatly to morbidity and mortality in this disease. Impaired T cell function in B-CLL has been suggested to result from inhibitory effects exerted by the clonal B lymphocytes. Thus, since the presence of leukemic cells may represent a major obstacle to efficient T cell activation, we isolated T lymphocytes from 12 B-CLL patients, stimulated them with phorbol 12-myristate 13-acetate (PMA) plus ionomycin for 4 hours, and then cocultured them with autologous leukemic B cells in a 1:1 ratio for 24-40 hrs. We found that CD86 and CD95 expression could be markedly upregulated using this approach ($p<0.0001$ vs. cocultures of CLL B cells and unstimulated T cells), whereas CD80 expression was augmented only in a minority of patients ($p=0.051$); more importantly, these effects were observed even when preactivated T cells were rechallenged with CLL B cells at the same low T/B cell ratio observed in vivo. CD40L was not the only determinant of the observed effects, since supernatants from preactivated T lymphocytes were capable to induce upregulation of the investigated markers on CLL B cells with high efficiency. Intracellular cytokine staining of preactivated T cells and blocking experiments with specific monoclonal antibodies identified IFN-γ as the cytokine mainly responsible for the above effects. Research is ongoing to assess the ability of CLL B cells with high expression of CD86 and CD95 to prime T cell responses and to undergo apoptosis, respectively.

**PO016**

LOW-DOSE FLUDARABINE IN ELDERLY PATIENTS WITH B-CLL REFRACTORY TO CHLORAMBUCIL TREATMENT

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In the last years the treatment of patients with chronic lymphocytic leukemia (CLL) has changed because of the introduction of new agents mainly the purine analogs. Fludarabine, alone or in combination with other drugs, has been reported to be effective in the treatment of CLL, both as first line and salvage therapy, but a relevant number of infectious complications have been described, particularly in elderly patients. The aim of this work was to evaluate the efficacy, the toxicity, and the incidence of infectious episodes of a regimen with low doses of fludarabine in elderly patients with B-CLL refractory to chlorambucil therapy. We report our experience: ten patients with refractory B-CLL with a median age of 73 years (4 in stage B and 6 in stage C) were enrolled. Fludarabine was administered by intravenous infusion at a dose of 25 mg/m² (max 50 mg) for 3 days every 4 weeks until a maximum of 6 cycles. All patients enrolled were evaluable for response. Two out of 10 patients achieved a complete remission (CR), 6/10 a partial response (PR) with an overall response rate (CR+PR) of 70%, according to National Cancer Institute-Working Group response criteria. Two patients were considered resistant. In only one patient, a severe neutropenia (neutrophils $<0.5 \times 10^9/L$) occurred and a pulmonary complication developed which required treatment with systemic antibiotics and granulocyte colony-stimulating factor (G-CSF). Non-hematologic toxicity was negligible in all patients. The low-dose fludarabine treatment appeared to be effective in this subset of B-CLL patients, reproducing a similar overall response rate obtained with other combination therapies based on fludarabine-full doses. In addition, in this group of elderly patients, toxic side effects were negligible and infectious complications very low.

**PO017**

FLOW CYTOMETRIC EVALUATION OF CYTOKINE PRODUCTION IN B-CLL PATIENTS

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Introduction. The study of cytokine production (CP) has been proposed by several Authors to follow the outcome in B-CLL. In particular, IL-6 and IL-10 levels have been associated with unfavorable characteristics
and worse prognosis. Since Flow Cytometry allows the simultaneous evaluation of multiple analytes (IL-2, IL-4, IL-10, IL-6, TNF-alpha and IFN-gamma), in this study we have extended the range of cytokine analysis, with the aim to verify a new test for the clinical management of B-CLL patients (pts). 

Methods. The study group consisted of 20 pts with diagnosis of B-CLL (10 pts in RAI 0, 8 pts in RAI 1, 2 pts in RAI 2). All the pts were off treatment for at least 3 months. The initial treatment was chlorambucil in 2 pts and CHOP in 1 case. Clinical and laboratory evaluation included medical history, physical examination, blood and platelet counts, serum chemistry, beta 2 - microglobulin, serum protein electrophoresis, quantitative immunoglobulin levels, peripheral and bone marrow lymphocyte immunophenotype, chest X-ray and abdomen and pelvis echoscan. CP was performed in peripheral blood samples by Cytometric Bead Array (CBA, BD Pharmingen - San Diego, CA), according to the manufacturer instructions, time 0 and every 6 months afterwards, and analysed by FACSCalibur Flow Cytometer - BD, using CBA Software. Phenotypic features were investigated by the following MoAbs: anti-CD19 PE, - kappa, -lambda, -CD5, -CD23, -CD11c, -CD38, -CD103, -CD10 and FM C7 FITC by 2 colours flow cytometry and Cell-Quest software - BD. As contro group the peripheral blood of 20 healthy donors was used. Statistical analysis was performed by Student's t-test. Results. The pts of this study were divided in 2 subsets, according to the CP levels: subset 1 included pts with CP < CP medium value of control group; subset 2 included pts with CP > CP medium value of control group. The most interesting data concerned IL-6 and IL-10 cytokines: subset 1 pts showed the clinical features of B-CLL early stages (9/10 pts in RAI 0, 3/8 pts in RAI 1); by contrast subset 2 pts resulted in more advanced stages (1/10 pt in RAI 0, 5/8 pts in RAI 1, 2/2 pts in RAI 2). Moreover we found a positive statistical correlation between beta 2-microglobulin levels and IL-6 CP (p < 0.05) or IL-10 CP (p < 0.03) or CD 38 positive lymphocytes (p < 0.001). Discussion. B-CLL pts show heterogeneous clinical courses. Therefore new identifiers of clinical subsets with favorable versus poor prognoses would be very helpful for patient management. Several parameters have been proposed, such as lymphocyte doubling time, circulating levels of beta2-microglobulin, bone marrow histology, CD38 positive lymphocytes and cytokine production. The results of this study show the involvement of IL-6 and IL-10 on B malignant cell proliferation and confirm the role of beta 2-microglobulin and of CD38 expression as important prognostic factors in B-CLL. In conclusion, flow cytometric evaluation of CP could be considered a useful mean to investigate the progress of disease and to correlate clinical features with outcome markers.

B-cell chronic lymphocytic leukemia (B-CLL) is a malignant B-cell proliferation with a wide spectrum of clinical of disease, course and prognosis. Moreover, based on the mutational status of the variable region of the immunoglobulin genes, CLL is separable into two subtypes with different cellular origin and different clinical behaviors, unmutated CLL (UM-CLL) being associated with a significantly shorter survival than mutated ones (M-CLL). CD38, originally described as a T-cell antigen, is expressed in a wide range of cell types, including medullary thymocytes, germinal center lymphoblasts, plasma cells, monocytes, stem cells and endothelial cells. Although its function is unknown, CD38 antigen was used as a marker of lymphocytes activation as well as in the systematic classification of T or B cell malignancies. Recently, CD38 expression was proposed as a prognostic indicator in B-CLL. In particular, CD38 expression detected in more than 30% of B-CLL cells, appears to be related to a worse course of the disease. Moreover, some authors suggested that CD38 expression correlates with the mutational status of immunoglobulin. Nevertheless, there has been some controversy about the real impact of CD38 expression as an independent prognostic factor as well as a predictor of Ig mutational status. Here we report CD38 gene expression analysis using different and complementary techniques in order to obtain expression data at transcriptional and post-transcriptional levels. In particular, flow-cytometry expressed as percentage of CD38-positive CLL cells and antibody-binding capacity (ABC) as well as quantitative RealtimePCR were performed on 50 B-CLL cases in which the immunoglobulin mutational status was also determined. Higher levels of CD38 transcripts were significantly present in UM-CLL than M-CLL (p<0.001). Strictly correlations were not found analysing the same cellular populations by flow cytometry, in particular CD38 expression reported as percentage of CD38 positive cells poorly correlates with the Ig mutational status. No statistical correlation was also found between ABC quantitative CD38 expression and Ig status. Nevertheless, a strong correlation was found when UM-CLL were examined separately, a large number of these CLL display higher CD38 expression levels with both ABC and real-time PCR. In conclusion, quantitative analysis of CD38 transcripts is an efficient parameter able to distinguish M-CLL from UM-CLL.
B-cell chronic lymphocytic leukemia (B-CLL) follows heterogeneous clinical courses and several biological parameters have been found to predict the clinical outcome. High CD38 expression identifies a subgroup of B-cell chronic lymphocytic leukemia patients with a greater disease activity and a shorter survival. In particular, CD38 positive B-cells show several functional properties, including the propensity to undergo apoptosis. Increased levels of pro-angiogenic factors have recently been demonstrated to be related to a higher risk of disease progression in early stages of B-CLL. Moreover, these angiogenesis-related factors have also been demonstrated to correlate with Bcl 2 expression. The involvement of both factors in the apoptosis pathway as well as their prognostic role in B-CLL prompted us to investigate the possible correlation between CD38 expression and pro-angiogenic factors. We analysed serum levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in 30 untreated patients with early B-CLL and in 20 healthy controls. The mean age of B-CLL patients was 71 years-old (range 56-88) and all of them were at Binet stage A. The patients showed different CD38 expression levels, detected by flow cytometric analysis. In particular, according to the percentage of leukemic cells positive for CD38, patients were classified into two groups: those with less than 20% CD38+ (low expression) and those with 20% or more CD38+ (high expression). VEGF and bFGF serum concentration levels were evaluated by a quantitative ELISA technique. Serum levels of VEGF were not significantly elevated in B-cell CLL patients (mean 283.7 pg/mL, range 0-889 pg/mL) compared with control group (mean 242.8 pg/mL, range 0-701 pg/mL). On the other hand, serum levels of bFGF were significantly higher in B-CLL patients (mean 15 pg/mL, range 0-66.7 pg/mL) than in healthy controls (undetectable in all) (p=0.01). We did not found any relation between serum levels of VEGF or bFGF and CD38 expression. Furthermore, no significant differences were found in serum levels of VEGF as well as of bFGF between patients with CD38 high and CD38 low expression. These data indicate the absence of correlation between serum levels of pro-angiogenic factors and CD38 expression, at least in early B-CLL patients, and suggest that these two negative prognostic markers could be related to different pathways involved in disease progression. Further investigations are necessary to define if any relationship among serum levels of VEGF or bFGF and CD38 could be present in patients at advanced clinical stage and during disease progression.

**PO020**

**HEPATITIS B VIRUS INFECTION IN CLL PATIENTS TREATED WITH FLUDARABINE AND STEROID COMBINATION THERAPY**


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One hundred and sixteen chronic lymphocytic leukaemia (CLL) patients with advanced disease who received a first line (46 pts) or second line (70 pts) Fludarabine (Flu) and steroid combination therapy (Flu + PDN: 81 pts; Flu + PDN +alpha-IFN: 24 pts; FAND: 11 pts) were retrospectively analyzed to evaluate the prevalence, morbidity and mortality, and predisposing factors for the occurrence of hepatitis B virus replication. Prior to treatment, liver function was abnormal in 11 cases (9.5%) and hepatomegaly was present in 65 cases (56%). Four patients (3%) were HbsAg and HbeAg positive. A complete Ag/Ab HBV assessment was performed in 74 cases and 17 (23%) were HbcAb positive. Among the HbcAb positive cases, 6 were HbsAb negative. Five HbsAg and HBV-DNA positive hepatitis B virus infections (4%) were recorded. In all cases, the hepatitis could be related to the reactivation of a prior HBV infection and occurred in patients who were HbcAb positive and HbsAb negative before therapy. Three cases were related to the replication of a precore mutant HbeAg negative and HbeAb positive, 2 were observed early during chemotherapy after the first and the sixth course, while the third case was recorded 16 months from therapy. The other 2 cases were HbeAg positive and were observed after 10 and 12 months after chemotherapy. No fulminant cases were recorded. After hepatitis, 1 patient died because of CLL, 1 persistently HbsAb negative died because of liver failure 52 months later, while 3 patients recovered and seroconverted. Of the 4 HbsAg, HbeAg and HBV-DNA positive hepatitis B virus infections (4%) were recorded. In all cases, the hepatitis could be related to the reactivation of a prior HBV infection and occurred in patients who were HbcAb positive and HbsAb negative before therapy. Three cases were related to the replication of a precore mutant HbeAg negative and HbeAb positive, 2 were observed early during chemotherapy after the first and the sixth course, while the third case was recorded 16 months from therapy. The other 2 cases were HbeAg positive and were observed after 10 and 12 months after chemotherapy. No fulminant cases were recorded. After hepatitis, 1 patient died because of CLL, 1 persistently HbsAb negative died because of liver failure 52 months later, while 3 patients recovered and seroconverted. Of the 4 HbsAg, HbeAg and HBV-DNA positive patients, none developed an acute hepatitis during therapy, 3 responded to chemotherapy; of these, 2 were maintained with alpha-IFN (3 MUI three times weekly), while 1 received long-term steroid therapy because of thrombocytopenia; the last patient, refractory to therapy, died because of CLL and disseminated HVZ infection. In conclusion, in our series HBV infection was an uncommon complication of CLL patients who received Flu and steroid combination chemotherapy and could be related to the reactivation of the HBV in all cases. Our data suggest that CLL patients should be tested for HBV before implementing cytotoxic and immunosuppressive therapy in order to identify patients with risk factors for HBV reactivation. The close monitoring of liver function and the benefit of a lamivudine treatment or prophylaxis should be investigated in these patients.
Alemtuzumab has recently been used in the treatment of refractory or relapsed CLL, at 30 mg i.v. three times weekly for 12-16 weeks. In pretreated patients Campath produced a median 31% PR and 33% OR, despite 6-20% CMV reactivation, 5.4% bacterial infections, 3.4% Pneumocistis carinii, 2.7% mycosis. Based on the evidence that terminal half-life of Campath has not yet been defined and that its efficacy at a lower dose (10 mg s.c. three times weekly) was previously described only in a selected group of patients responsive to fludarabine treatment, we evaluated the efficacy, immunosuppression, related infection diseases and costs of using Campath at 10 mg i.v. three times weekly in pretreated CLL. Starting from November 2002, four CLL pretreated CLL patients, three relapsed after fludarabine treatment and one relapsed after the CHOP regimen plus auto-PBSCT, have been treated with Campath at 10 mg i.v. three times weekly. Three patients were male with a median age of 58.5 years; before starting treatment three patients were in stage B/II, and one patients in stage C/IV. In association with CLL, one male patient showed autoimmune hemolytic anemia and one immune trombocytopenia while the female patient was affected by acquired von-Willebrand disease. All patients received antibiotal prophylaxis with Acyclovir, trimetoprin-sulfamethoxazole and fluconazol from the start of treatment until two months after stopped treatment. At the time of the treatment the median of lymphocytes was 28,764/mmc (range 15,620-62,460) on peripheral blood (PB) and 67% on bone marrow (BM) aspiration; no patients showed marked lymphoadenomegaly. Follow-up consists of blood cell count before every administration, antigenemia and PCR for Cytomegalovirus weekly, and costs of using Campath at 10 mg i.v., but the data encouraging the use of such treatment in refractory or relapsed CLL patients.

Despite many reports regarding the comparison of lymphocyte recovery after autologous bone marrow transplantation with CD34 selected or unselected peripheral blood stem cells (PBSC) for HD, NHL, or multiple myeloma, no data are available concerning the immune recovery after PBSC transplant for CLL. The aim of the study was to evaluate lymphocyte recovery after autologous transplantation in two different lymphoproliferative groups of diseases: CLL group and lymphoma group (HD and NHL). We compared the immunological recovery of six patients affected by CLL (4 males, 2 females with a median age of 50 years) with ninety-nine patients affected by lymphoproliferative disorders (58 males, 41 females, with a median age of 38 years). We evaluated the lymphocytes recovery and the immunoglobulin assay before the transplant, on day 15, 30, 60, 90, 120. We studied: total lymphocytes, 5 subclasses of lymphocytes (CD3, CD4, CD8, CD19, CD16-56) and immunoglobulin (IgG, IgA, IgM) serum levels. The median number of CD34+ cells infused was respectively 2.57×10^6/kg (range 1.91-4.63) in CLL group and 6.05×10^6/kg (range 1.2-36) in lymphoma group. Lymphocyte engraftment after transplantation occurred promptly in the two groups. A lymphocyte count > 0.5×10^9/L was obtained on day +30 (range 15-60) in CLL patients, and on day +18.5 (range 9-64) in lymphoma patients (p=0.047). From day +30 lymphocyte count showed an increase reaching stable median values above 1×10^9/L from day 90 in both groups. Pretransplant T cells appeared to be comparable in the two groups with median values below the
normal range (0.73×10^9/L in CLL group and 0.57×10^9/L in lymphoma group). After transplant the recovery of CD3+ cells was similar in the two groups with a trend toward normalization, without reaching normal value during the study period. The count of CD4+ T helper lymphocytes showed values below 0.4×10^9/L before transplant. Starting from day +15 median values were constantly below 0.2×10^9/L in the two groups. CD8+ suppressor T lymphocyte during the first month, showed median values below the normal range in two groups. Thereafter lymphoma group achieved normal value on day +60 while CLL group on day +90. The persistent unbalance of CD4/CD8 ratio in the two groups was due to the high level of CD8+ and the concomitant reduction of CD4+. CD19+ B cells showed recovery starting on day +30 without achieving normal values until day +120. Immunoglobulin G levels in CLL group reached normal value, while, in lymphoma group, the levels were below the normal range; immunoglobulin A levels were into the normal range in both groups, while immunoglobulin M levels did not reach the normal range; these results did not achieve statistical significance. CD16/56 returned to normal values starting from day +30, when lymphoma group showed a value significantly higher than CLL group (p=0.027). In conclusion, the immunological recovery after PBSCT was similar in the two groups of lymphoproliferative disorders even if a longer follow up and a larger series of patients are necessary to assess the normalization of the immune system.

P0023

QUANTITATIVE MOLECULAR EVALUATION OF MINIMAL RESIDUAL DISEASE IN CLL PATIENTS: EFFICACY OF IN VIVO PURGING BY CAMPATH 1H

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The application of novel therapies, such as autologous or allogeneic stem cell transplantation, in the management of chronic lymphocytic leukemia (CLL) resulted in higher hematological response rates, but the complete eradication of tumor cells rarely occurs. Campath 1H is a humanized monoclonal antibody specific for CD52 antigen; it has been shown to be extremely effective in killing lymphocytes in vitro and in vivo, even in heavily pre-treated patients, resistant to fludarabine. This latter chemotherapeutic agent is able to induce 60% of overall responses, with 15% of CR, but rarely these responsive patients achieve molecular remission. PCR-negativity has reported to significantly improve PFS rate in CLL patients, but the in vitro purging procedures did not offer more than 15-20% of molecular remissions. In this study we employed a molecular semi-quantitative PCR method to assess the capacity of Campath 1H to induce PCR-negativities in eight patients already resistant to fludarabine. IgH rearrangement was co-amplified with a housekeeping gene and PCR products analyzed by a DNA automatic sequencer. This method allows us to precisely measure and compare peak areas, thus offering a quantitative evaluation of the minimal residual disease (MRD). Each patient was evaluated at diagnosis, after fludarabine and after Campath 1H treatment. Before therapy with the anti-CD52 antibody, six of the eight cases were resistant to fludarabine and the remaining two were persistently PCR-positive. The median interval between the last therapy course and the start of Campath 1H therapy was 14 weeks. Patients received Campath 1H subcutaneously, three times at week for six weeks, in escalating doses up to 10 mg, with a median dose of 180 mg. After therapy with fludarabine, only one patient (12.5%) achieved molecular remission; in two cases the IgH quantity increased >1log, in other five patients MRD appeared stable and in only one decreased >1 log. After treatment with Campath 1H, two patients achieved the molecular remission (25%), but, interestingly, in four of the five cases quantitatively evaluated the IgH rearrangement decreased >1 log. With a median follow-up of 16 weeks after treatment with the anti-CD52 antibody, we observed 72% of hematological responses, with 43% of CR. A significant reduction of lymph node and spleen diameters was noted in the 40% of patients. All cases were evaluable for toxicity: a skin reaction in the site of the subcutaneous injection, of grade 2 according to the WHO scale, was observed in two and fever in four cases. As regards hematological toxicity, two patients developed grade 3 neutropenia and two cases hemolytic episodes. Two patients showed CMV and one VZV reactivation. These results show that Campath 1H is a quite safe therapy for CLL patients. Interestingly, it resulted active also in patients resistant to fludarabine and it seems to represent an efficacious in vivo purging tool, either by significantly reducing the MRD or improving molecular remission rates.
To investigate whether surgical resection with/without radiation therapy is safe and effective for patients with low-grade gastric MALT lymphoma. Sixty-seven patients with IE-IIE stage disease low-grade gastric MALT lymphomas according to Musher's staging system were submitted to combined-modality treatment including surgery with/without radiation therapy between January 1986 and December 1998. The strategy was always involved complete resection of the primary tumor: all patients underwent gastrectomy (38 subtotal and 29 total). Complete surgical staging was based on three pathological features: disease confined within or beyond the serosa, negative/positive regional lymph nodes and negative/positive surgical margins. Patients who presented at least one of three pathological factors were treated with adjuvant radiation therapy after resection. Tumor-free margins were obtainable with subtotal gastrectomy in 38 (57%) patients. At surgical staging, 39 (57%) patients presented at least one of pathological feature and were submitted to radiation therapy; 10 (15%) patients who had both intramural invasion and lymph node involvement received whole abdominal irradiation. The complete response rate was 98%. Only 5 (7%) patients relapsed (at 24, 66, 74, 78 and 148 months, respectively). No operative death was recorded. At 180 months, the overall actuarial survival and 148 months, respectively). No operative death was recorded. At 180 months, the overall actuarial survival and 15-year relapse-free survival is 92% and the projected 15-year relapse-free survival is 83%. The present report suggests that surgical treatment with/without radiation therapy is a safe and effective option for patients with stage IE-IIE low-grade gastric MALT lymphomas.

The optimal treatment for gastric diffuse large B cell lymphoma (GDLBCL) is still controversial. Surgery, radiotherapy (RT) or chemotherapy (CT) are the main options as first line treatment. In particular CT alone is considered one of the possible treatment for patients with high grade gastric non Hodgkin lymphoma (NHL). However the lack of studies with large number of patients with homogeneous characteristics make this modality treatment still experimental. The aim of present retrospective analysis carried out in two institutions (European Institute of Oncology and Catholic University of Sacred Heart in Rome) is to evaluate feasibility, toxicity and clinical results in GDLBCL treated with CT alone. Forty eight patients (23 male, 25 female) with 58 years median age (range 29–81), treated in two institutions from 1990 to 2002 were reviewed. The majority of patients had a good performance status at diagnosis with low IPI. All patients underwent endoscopy and echoendoscopy as diagnostic procedure and subsequently were staged according to international procedures (CT scan, bone marrow biopsy, histology and biochemistry examinations). All 48 patients received anthracyclin containing regimen (CHOP or CHOP-like 31 patients, ProMACE-CytaBOM 3 patients, MACOP-B 5 patients and VNCOP-B 9 patients). Number of cycles administered range from 4 to 6. Only 18/48 patients reduced or delayed treatment because of mild (G2) or in few case severe (G3-G4) hematologic or non-hematologic toxicity. Only one patient underwent gastrectomy because of gastric perforation. Forty three patients on 48 achieved a pathological complete remission (pCR), 3 pts a partial remission, 1 patient stable disease while only one patient progressed after two cycles of CT. With median follow-up of 2.5 years only 5 patients relapsed. Our results suggest as CT may be considered as first option for GDLBCL since the low rate of complications and the relevant rate of pCR recorded. Clinical results need confirmation in a randomised trial which compare CT alone versus RT which may consider as standard treatment. Such study is now ongoing on behalf of the International Extranodal Lymphoma Study Group.

Distinct types of lymphoma may develop in the same patient both simultaneously and sequentially. The term composite lymphoma had been included in the Work-
ing Formulation to denote such event, but in the more recent classifications (REAL and WHO) it was not considered as a specific category. By retrospectively analyzing the series of patients affected by lymphoma consecutively seen at our Institution in the last eight years, we identified 56 patients with at least two different diagnoses of lymphoma. Cases were reviewed by a team of pathologists according to the WHO criteria and 17 of them were excluded because an unifying diagnosis could be done according to morphologic, phenotypic and clinical parameters. The 37 confirmed cases were defined as multiple lymphoma and subdivided into three different categories: 1) composite lymphoma, when both lymphomas were present at the same time in the same tissue (3 cases); 2) simultaneous lymphoma, when different histologies were found in different tissues at the same time (3 cases); 3) sequential lymphoma when they were diagnosed at different times (31 cases). Lymphnodes and bone marrow were the most frequently involved disease sites. Composite lymphoma included a combination of lymphocytic lymphoma and Hodgkin disease in two cases and follicular lymphoma in one case, respectively. Histologic combinations in simultaneous lymphoma included mycosis fungoides and marginal cell lymphoma, lymphocytic lymphoma and anaplastic large cell lymphoma; follicular lymphoma and Hodgkin disease in one case each. Sequential lymphomas included a combination of lymphomas of different immunophenotype in 6 cases (Hodgkin and NHL in 3; anaplastic/null and B NHL in 2; T and B NHL in 1 case) and of two types of B cell lymphoma in 25 cases. Two different indolent histologies and two different aggressive histologies were found in 3 and one case, respectively. The remaining 21 cases showed a combination of an indolent followed by an aggressive B cell lymphoma. Molecular analysis by PCR using primers for the J-region of the IgH gene was performed in 16 of these 21 cases. Amplification bands could be obtained in 13 samples; the same clonal IgH rearrangement was confirmed in 11/13 cases, pointing to histologic transformation of the lymphoma clone as the most frequent pathogenetic mechanism of sequential multiple lymphomas. One case was unclear and one showed a polyclonal pattern. Molecular analysis of the other subgroups of multiple lymphoma is currently ongoing. The clinical features of multiple lymphomas were compared with those of lymphomas of a single histologic type. No significant differences in age, sex, stage, IPI score and response rate were found. Multiple lymphomas showed worse actuarial disease-free and overall survival, which was statistically non significant. We conclude that further studies are needed to better delineate the different pathogenetic mechanisms of multiple lymphomas, a group of diseases which is not uncommon and may have a bad prognosis.

**P0027**

**CLINICAL AND PROGNOSTIC FEATURES OF PATIENTS WITH LIMITED STAGE DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE STUDY PERFORMED BY THE ITALIAN LYMPHOMA INTERGROUP**


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Introduction: The evaluation of prognostic factors such as the International Prognostic Index (IPI) in patients with diffuse large B-cell lymphoma (DLBCL) may be valuable in selecting treatments. However, few studies have analyzed these factors in patients with localized disease and the definition of a prognostic model is still controversial. The ILI has performed a retrospective study with the aim to assess clinical behavior and evaluating prognostic features of this subset of patients. Patients and methods: 1,637 patients with a localized (Ann Arbor stage I-II) aggressive B-cell lymphoma (WF: G or H, WHO:DLBCL) diagnosed from 1988 to 2002 were registered by 4 Italian cooperative groups and 2 single institutions; the 993 cases with fully evaluable data are the subject of this preliminary analysis. Results: The characteristics at presentation are the following: median age 59 years (range, 14-91), M/F ratio, 1.08. Clinical stage was I in 172 (17%), II in 290 (29%), III in 217 (22%) and IIE in 314 (32%) patients, respectively. Extranodal involvement occurred in 604 (61%) cases with 67 (7%) cases presenting with >1 extranodal sites. Bulky disease (>10 cm) and ECOG-PS >1 were present in 23% and 14% of patients, respectively. Abnormal biochemical data included: an elevated LDH (26%), β2-microglobulin (25%) and ESR (40%) and a reduced albumin (< 3.5 g/dL) in 22% of the cases. According to modified-IPI (MIPI) including stage II adverse features, 19% of cases had >2 risk factors. Treatments varied in different institutions at the time, but in the majority of cases were anthracycin-containing regimens. After a median follow-up of 38 months (range 1-183 months), 3 and 5-year OS rates were 75% and 72%, respectively. By univariate analysis the following 8 variables and MIPI (p<0.0001) are predictive of a short survival: age >60 yrs (P=0.005), stage II (p<0.01), ECOG-PS>1 (p<0.0001), B symptoms (p=0.0002), bulky disease (p<0.0001), ESR >30mm (p=0.0007), elevated LDH (p<0.0001) and reduced albumin (<3.5/g/dL: p=0.0001). Conclusions: This preliminary analysis of 993 patients with localized DLBCL identified pretreatment characteristics predictive of survival. Features that will remain
individually significant in multivariate analysis will be incorporated into a prognostic model which after validation in the remnant sample will be helpful in the selection of appropriate therapeutic approaches in individual patients.

PO028
VINORELBINE, GEMCITABINE, PROCARBAZINE AND PREDNISONE (ViGePP) REGIMEN AS SALVAGE THERAPY IN RELAPSED OR REFRACTORY AGGRESSIVE NON-HODGKIN'S LYMPHOMA: A PHASE II STUDY OF GISL (GRUPPO ITALIANO PER LO STUDIO DEI LINFOMI)
on behalf of GISL (Gruppo Italiano per lo studio dei Linfomi)

Background: Patients (pts) who fail to achieve complete remission or relapse after initial therapy for aggressive non-Hodgkin's lymphoma (NHL) have a poor prognosis. Better results have been reported with high-dose therapy (HDT) and stem-cell rescue in patients with chemosensitive relapse. However, many patients are ineligible for this potentially curing approach because of age or associated morbidity. Therefore, newer combinations, including agents with proven efficacy, without cross-resistance, and not included in frontline regimens are desirable. Vinorelbine and gemcitabine are both relatively new drugs of proven effectiveness in aggressive NHL when given as single agents. To evaluate the efficacy and safety of gemcitabine and vinorelbine combined with procarbazine and prednisone in the treatment of pts with relapsed or refractory aggressive NHL. Patients and methods: From November 1999 to September 2002, 69 pts with aggressive NHL including Grade III follicular (3), MCL (2), diffuse large cell lymphoma (54), Burkitt's lymphoma (1) and T-cell lymphoma (9) were enrolled at different GISL Institutions. Twenty-five pts (38%) had refractory and 41 (62%) relapsed disease. The median age was 64 years (range: 25-79) with 71% older than 60 years; 75% of patients had Stage III or IV disease, 66% extranodal involvement and nearly half of them (45%) were at intermediate-high risk according to IPI. LDH level was higher than normal in 55% of pts. ViGePP schedule consisted of the administration of VNR (25mg/m2 IV, dd 1,8), GEM (800 mg/m2 IV, dd 1,8,15), PCZ (100 mg/m2 PO, dd 1-7) and PDN (60 mg/m2 PO, dd 1-15) every four weeks for six courses unless progression of disease. Results. At time of present analysis 66 pts were assessable for response: 14 pts (21%) achieved a CR and 12 (18%) a PR with an ORR of 39%. Response rate was 54% for pts with normal LDH (p=0.07) and 45% for pts with good performance status. At March 2003, after a median follow-up of 21 months (range: 6-36 mos), 21 pts (32%) were alive, 8 in CR, and 45 (68%) deceased, most of them of disease progression. The OS at 30 months was 33%. A statistically significant better survival was observed in pts with normal LDH (p=0.03), good performance status (p=0.01) and, obviously, in those responding to ViGePP (0.0003). Toxicity was mainly hematologic with WHO grade 3-4 neutropenia in 49%, thrombocytopenia in 20%, and anemia in 25% of cases. Conclusions. Considering the characteristics of pts treated in this study, ViGePP has to be considered an effective salvage treatment for pts with aggressive NHL. Better results have been observed in pts with more favorable clinical features prior to treatment.

PO029
UPFRONT TREATMENT WITH HIGH-DOSE SEQUENTIAL CHEMOTHERAPY AND AUTOGRAFT GIVES PROLONGED SURVIVAL TO LOW-GRADE LYMPHOMA PATIENTS: A TWELVE-YEAR EXPERIENCE
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High dose sequential chemotherapy regimens with autologous transplantation have been frequently employed in young patients with low-grade lymphoma. This treatment proved successful both in terms of clinical response and short-term outcome. However data after a very prolonged follow-up are still lacking. In the present abstract we present the clinical results of our single Center experience which started in 1990. Fifty-five patients, aged 65 or less have been enrolled in this trial: 15 had small lymphocytic lymphoma (SLL), 40 had follicular lymphoma (FL) and 10 of them with signs of histologic transformation (t-FL). I-HDS included: (1) tumor debulking, by 2 APO + 2 DHAP courses; (2) sequential administration of high-dose (hd) etoposide, methotrexate, and cyclophosphamide, followed by peripheral blood progenitor cell (PBPC) harvest; (3) hdmitoxantrone + melphalan with PBPC autograft. There were 4 treatment-related deaths. Late toxic episodes occurred in 3 patients (5,4%) and consisted of a myelodysplastic syndrome (MDS) and two solid tumors (one rectal cancer and one head and neck cancer in a heavy smoker). Two FL had disease progression, three SLL achieved a stable PR. Overall, 46 patients (83,6%) achieved CR. At a median follow-up of 6 years, the estimated 12-year OS was 76%. OS was not significantly different between SLL and FL. Interestingly estimated EFS at 12 years was 48%, while time to next treatment was 68%. These results indicate that hd-approach in
Primary non-Hodgkin’s lymphoma of bone (PLB) is a rare clinico-pathological entity. Consequently it is difficult to identify major prognostic factors and optimal treatment for this malignancy. We present a single institution’s experience, at Catholic University of Sacred Heart in Rome, from 1996 to 2002, concerning PLB therapy. A retrospective review was done of 20 patients with PLB. Diagnosis was performed on open bone biopsy. The male to female ratio was 2.3. The median age at onset was 63 years (range 20-84 years). At onset 7 patients presented stage IE (35%), 13 stage IVE (65%), 2 B symptoms (10%), 18 a large B cell lymphoma (90%) and 2 a small B cell lymphoma (10%). The most affected sites were vertebral (8 patients) and femur (4 patients). The median patients follow-up was 2.5 years (range 0.4-6.2 years). Four patients (20%) died for relapse or disease progression. One patient (5%) died for therapy related cause and one (5%) for a different cause. All patients were submitted to third generation chemotherapy protocols for NHL. Secondary 17 patients received radiotherapy (RT). Three patients were not submitted to RT because of disease progression. Nine patients with age >60 years, with a good performance status (PS) or without important coexisting disease were submitted to the ProMAC-ACE-CytaBOM intensified chemotherapy regimen. Five patients with age >60 years, with a bad PS and coexisting important disease were submitted to the VNCOP-B chemotherapy regiment. Six patients with age < or = 60 years, without coexisting important disease and with good general conditions were submitted to the MACOP-B chemotherapy regimen. Survival curves were calculated by Kaplan-Meier method and risk assessment was performed by Odds Ratio (OR). In our study, analyzed data never reached statistical significance, probably because of the small number of patients of our cohort. Patients treated by the MACOP-B had a 5-years overall survival higher (83.3%) than other patients (66.7% for patients treated by the ProMAC-ACE-CytaBOM and 75% for patients treated by the VNCOP-B). Patients treated by VNCOP-B had a better 5-years disease free survival than literature’s patients treated by the CHOP regimen (80% vs 55%). The ProMAC-ACE-CytaBOM regimen seems to produce a bad 5-years event free survival (33% vs 50% and 60% for MACOP-B and VNCOP-B respectively) and a greater toxicity (OR 2.5) if compared to other used chemotherapy regimens. Among various tested parameters, only LDH >400, dorsal vertebrae localisation, presence of B symptoms and appearance of new bony lesions within 2.5 months from diagnosis, seem to have a bad prognostic value in our study. Two patients treated by cement with antiblastic drugs positioned in affected bony sites are currently in complete remission. Nevertheless only larger numbers of patients and multicenter studies will be able to characterize better the major prognostic factors and the appropriate therapeutic options for this rare disease.
was 30.6 years (range 16-73). The histologic subtype was nodular sclerosis in 22 patients, mixed cellularity in 5 patients, lymphocytic depletion in 1 patient. According to the Ann Arbor staging, 21 patients were stage I, 5 stage III and 2 stage IV. Nineteen patients had been treated with chemotherapy (ABVD) and radiotherapy while nine patients with chemotherapy alone. Results: CT was negative in 13 patients, three out of them recurred while ten were free from relapse after a median follow-up of 29 months (negative predictive value NPV = 77%). In the other 15 patients CT was positive and only one patient relapse with a positive predictive value (PPV) = 7%. The FDG-PET was negative in 20 out 28 patients, eleven out of these 20 patients had residual mass at CT scan. None of the 20 patients relapsed after 25 months average follow-up (range 10-41), and the NPV = 100%. FDG-PET was positive in 8 patients (29%); four out of these 8 patients relapsed (PPV = 50%), while 4 were free from disease after 27 months average follow-up (range 12-34 months). The presence of residual mass was less consistently associated with poor prognosis than positivity of PET. In fact, only one patient out of 15 (7%) with positive CT relapsed vs 4 patients out of 8 (50%) with positive PET. The worst prognosis of patients with positive PET is confirmed from results of EFS with 42% vs 68% of patients with positive CT at 520 days. Conclusions: our data act in favour of adding FDG-PET in the non invasive assessment of HD with post treatment residual masses. High sensitivity and NPV of FDG-PET scan along with good prognosis of FDG-PET negative patients may lead to modify clinical management. Prognosis with a negative PET is very good and we have avoided overtreatment. In the the case of a positive FDG-PET, even in agreement with conventional CT, a close follow-up with repeated CT or ultrasound studies, antibiotic therapy and eventually biopsy is suggested to avoid false positive results.

PO032
IS BONE MARROW TREPINE BIOPSY ALWAYS MANDATORY IN HODGKIN’S DISEASE?
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Introduction: Staging investigations performed at diagnosis for patients (pts) with Hodgkin’s disease (HD) are important to determine the correct treatment and prognosis. Recommended procedures for staging include bilateral bone marrow trephine biopsy (BMM TB). The aim of this study was to determine whether BMM TB is mandatory for all categories of pts. affected by HD. Patients and methods: we have retrospectively examined data from 562 pts treated from 1994 to 2003 in our institution and included in HD94 intermediate (389 pts) and advanced (173 pts) stage protocols. All pts were submitted to Rye staging system that include BMM TB. Results: of the 562 pts, 28 (5%) had bone marrow involvement (BMMI). 14 patients (50%) had a monolateral BMMI and 14 patients (50%) a bilateral BMMI. Of these 28 pts, 26 (93%) were already clinically staged as advanced stage (22 pts stage III, 4 pts stage IV) while 2 pts (7%) as intermediate stage (stage IB). Of the 562 pts, no patients clinically staged as I-IIA showed positive BMMI TB. We compared the 28 pts with BMMI (group A) to the other 145 pts in advanced stage without BMMI (group B) and the 389 pts in intermediate stage. At diagnosis there were not differences between two advanced groups as concerns erythrocyte sedimentation rate (ESR) (median 70 in both groups), median age (34, range 21-62 and 32, range 20-68 years), M/F ratio (16/12 and 67/78), symptoms (82% and 85%), respectively in group A and B. Regarding intermediate HD pts, median ESR was 43 and 47% of pts were symptomatic. According to data reported in literature, mixed cellularity histopathologic subtype was more frequent in pts with BMMI (10 cases; 35.7% vs 17.9% in group B vs 8% in intermediate stage). Considering extra-nodal sites, of the 63 patients staged as IV without BMMI, 45 had lung, 10 pleura and 8 liver involvement; of the 28 patients with BMMI 2 patients had lung, 1 patient liver and 1 patient humerus involvement. Discussion: BMMI TB is currently recommended for staging pts affected by HD. However, many studies have shown that BMMI is not, by itself, an adverse prognostic factor, and does not define a special high risk group requiring a different therapeutic approach. In our study we did not find differences in terms of results between the two groups of advanced stage pts, but the low number of patients hampers firm conclusions. In our study 92.8% of the patients with BMMI were already clinically staged as advanced stage so their treatment plan did not change on the basis of the bone marrow results. Of the 28 pts only 2 were clinically staged as initial stage, so only in 2 pts the BMMI TB was determinant and changed stage and treatment plan. Both pts, though initially staged (I), were symptomatic (fever) with high ESR. In accord to other reports we found that 50% of patients had a monolateral BMMI. Because of the focal nature of HD involvement, when necessary, BMMI TB would be done. These data suggest that in patients clinically staged as I-IIA BMMI TB is not necessary. However, BMMI TB is recommended in the presence of B symptoms also in patients with localized stage disease. Furthermore, both magnetic resonance imaging and 18-F-fluorodeoxyglucose-positron emission tomography (FDG-PET) have recently shown encouraging sensitivity and specificity for the detection of BMM with HD.
Patients with aggressive NHL who have relapsed after ASCT or refractory to salvage therapy have a very poor prognosis. The association of Rituximab (RTX) with CHOP obtained good results in this population, but its role as salvage treatment has not been elucidated yet. In our preliminary experience, we assessed the efficacy and the tolerability of a new combination of CHOP+RTX including the GM-CSF both in order to upregulate the CD20 expression in vivo and to increase the ADCC mediated lysis. We also wanted to add the GM-CSF to CHOP-RTX in order to improve the hematologic tolerance in this population. We treated 34 patients affected by aggressive lymphoma relapsed after ASCT or refractory to more than two lines of chemotherapy by a combination of CHOP-RTX and GM-CSF. The population was composed by 15 male and 19 female; the pts were affected by aggressive NHL (18 DLCL, 8 transformed HG NHL and 8 mantle NHL); thirty pts were in stage III-IV and 23 had relapsed after autologous and 1 after allogeneic BMT. Their median age was 62 years (28-79), performance status was 0-2 in 29 pts and 3-4 in 5 pts (WHO); median number of previous CHT regimens was 3 (1-7); the disease status was SD, relapse and progression respectively in 4, 23 and 7 pts. We performed 171 infusions of RTX and CH/T with a very low incidence of grade III-IV toxicity: 1.2% of acute events, 4.7% of extra hematological toxicity but a not negligible hematologic toxicity (27.5%). The ORR in the 31 evaluable pts was 60%, with 58% of CR; 16 pts (53%) are still in CCR and 2 with stable disease with a median follow up of 14 months (2-36). In conclusion the treatment with CHOP-RTX-GM-CSF is feasible and effective also in this group of heavily pre-treated pts refractory to other treatment or who have relapsed after ASCT.

First line anthracycline based chemotherapy results in a stable control of the disease in about 50% in patients with aggressive non Hodgkin’s lymphoma (NHL). Alternative strategies are clearly needed in order to improve therapeutic results in patients who early relapse or never achieve complete remission (CR). In particular, high dose chemotherapy followed by autologous stem cell transplantation (ASCT) can be effective in this setting. On this basis, an ideal salvage regimen should be able to induce a substantial reduction of the tumour burden as well as an effective mobilization of peripheral blood stem cells (PBSC). Preliminary data indicate that ifosfamide-based regimens induce high response rate with successful PBSC mobilization in a considerable number of poor risk NHL patients. Here we report our experience with a combination of ifosfamide, epirubicin and etoposide (IEV) from a series of 26 patients (17 males and 9 females, median age 45 years, range 19-70) with primary refractory (n=5), partially responders (n=10) or early relapsing (n=11) high grade NHL. At diagnosis, 4 patients were in stage II, 8 in stage III, 14 in stage IV; 18 patients had IPI score 0-2, 8 had IPI / 3. Twenty-one patients had diffuse large B-cell lymphoma and 5 lymphoblastic lymphoma. According to IPI score, at diagnosis 20 patients had received MACOP-B and 6 CHOP. Four patients had been given a further salvage regimen before IEV. Treatment consisted of epirubicin 100 mg/m²/iv on day 1, ifosfamide 2500 mg/m²/iv on day 1 to 3, etoposide 150 mg/sqm/iv on day 1 to 3. Each patient was programmed to receive two courses. CD34+ cells were monitored at hematological recovery after the first course and apheresis performed when CD34+ cells were>20/µL. Following IEV administration, 11/26 patients (42%) obtained CR: among these 2 were primary refractory, 6 were relapsed and 3 converted from partial remission (PR) to CR. PR was achieved in 7/26 patients (27%), with an overall response rate of 69%. PBSC mobilization was successful in 24/26 patients (92%) with a median collection of 15,6±10/kg (range 5,6-50) CD34+ cells. Hematologic toxicity was characterized by grade 1-2 neutropenia in 5 patients and grade 3-4 in 21; grade 1-2 thrombocytopenia occurred in 11 patients, while grade 3-4 in 15 patients, platelet administration being necessary in 5 cases. Packed red cell transfusion was needed in 9 cases. There was one infectious episode by Herpes Zoster, while FUO requiring empiric intravenous antibiotic treatment was needed in 6 patients. Finally one patient experienced acute pancreatitis. Twenty-four out of 24 did actually receive ASCT, while, between those who failed to mobilize, one patient is in continuous CR after 2 additional courses of IEV and one died of progressive disease. After ASCT, 6 further CRs were obtained, and 15 out of 26 patients are in continuous CR at the time of writing (median follow up:12 months, range 6-28). We conclude that IEV is an effective salvage regimen for refractory/relapsing patients with NHL. Mobilization is successful in the vast majority of the cases with highest feasibility of ASCT.
CLINICOPATHOLOGIC STUDY
IN KIDNEY TRANSPLANT RECIPIENTS: A MONOCENTRIC
PO035
POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS
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Organ transplant recipients receive immunosuppressive
drugs to prevent graft rejection; however, due to
immunosuppression, transplanted patients are at higher
risk of developing lymphoproliferative disorders than
general population. Therefore, post-transplant lympho-
proliferative disorders (PLTD) represent a troublesome
complication of organ transplantation. Between November
1996 and November 2002, sixteen cases (10 male
and 6 female) of PLTD, out of 793 kidney graft recipients,
were referred to our Institution. Median age at diagno-
sis was 56 years (range 23-69), and the median time
between kidney transplant and PLTD diagnosis was 57
months (range 8-378). Only two patients had polymor-
phic PLTD, the remaining being monomorphic PLTD (10
DLC, 2 Burkitt, 1 anaplastic, 1 immunoblastic, 1 plas-
ma blastic, 1 multiple Mieloma). Sixty-nine percent of
patients showed advanced stage (III-IV) lymphoma, 45%
B symptoms, 76% extra-nodal involvement and 69%
intermediate/high and high IPI score. Epstein-Barr virus
genome was detected in 7 cases and HHV-8 in one case.
The first step of treatment program consisted of reduc-
tion of immunosuppressive therapy; thereafter, eight
patients received combination chemotherapy, five
patients anti-CD20 monoclonal antibody (Rituximab),
while two patients died before any treatment was start-
ed. Graft rejection occurred in two patients. As comple-
tion of treatment, two patients received radiotherapy,
and two patients received high-dose therapy and periph-
eral stem cells infusion. After immunosuppression reduc-
tion, two patients died rapidly for progression of aggres-
sive lymphoma; among chemotherapy-treated patients
(n=8), two died for progression, one for toxicity, and five
obtained a clinical response (CR/RP). Two of the respond-
ing patients died for relapse, while the others are alive.
Among patients treated with Rituximab (n=5) one had
progression, while the others obtained a durable response
(CR/RP). Overall survival was 47.7% with a median fol-
low-up of 32 months (range 1-70). In conclusion, PLTD
represent a worrisome complication of organ transplan-
tation, because of its aggressive presentation and high
risk of progression/relapse. Even if different therapeutic
approaches are feasible, treatment strategies are need-
ed to better control the disease and overcome chem-
resistance.

PO036
COMPLEMENT ACTIVATION DETERMINES THE THERAPEUTIC
ACTIVITY OF RITUXIMAB IN VIVO*
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Rituximab is an anti-CD20 chimeric monoclonal antibo-
dy effective for the treatment of B-NHL. It can lyse
freshly isolated lymphoma cells as well as cell lines in vit-
or through both complement and antibody dependent
cellular cytotoxicity, and has some pro-apoptotic activi-
ty. The mechanism of action of rituximab in vivo is how-
ever still a matter of debate. We have set up a new in vivo
model in non-immunodeficient mice by stable transduc-
tion of the human CD20 cDNA in the murine lymphoma
line EL4. Syngeneic C57/BL6 animals injected i.v. with
8×10^4 CD20^+ lymphoma cells died within 30 days with
evident hepatic, renal and bone marrow involvement.
Immunohistochemistry and PCR analysis confirmed the
presence of EL4-CD20^+ cells in these organs. A single
injection of rituximab or the murine anti-CD20 antibody
1F5, given i.p. one day after the tumour, cured 100% of
the animals. Indeed, at week four following tumour cell
inoculation, CD20^+ cells were undetectable in all organs
analysed in rituximab treated animals, as determined by
immunohistochemistry and PCR. Rituximab had no direct
effect on tumour growth in vitro. Depletion of either nat-
ural killer cells or neutrophils or both in these animals did
not affect the therapeutic activity of the drug. Similarly,
rituximab was able to eradicate tumor cells in athymic
nude mice, suggesting that its activity is T cell-indepen-
dent. In contrast, the protective activity of rituximab or
the 1F5 antibody was completely abolished in syngene-
ic knock out animals lacking C1q, the first component of
the classical pathway of complement (C1qα−/−). These
data demonstrate that complement activation is a fun-
damental step of rituximab therapeutic activity and sug-
gest novel strategies to improve rituximab activity in vivo.

PO037
BONE MARROW INFILTRATION PATTERN IN SPLENIC MARGINAL ZONE
LYMPHOMA WITH OR WITHOUT CIRCULATING VILLOUS
LYMPHOCYTES
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Splenic zone lymphoma (SMZL) with or without circulating villous lymphocytes (VL+/VL-) is listed as a definite entity in the WHO classification. This lymphoma is believed to involve prevalently if not exclusively the spleen and peripheral blood. We studied the frequency, degree and pattern of the bone marrow infiltration at diagnosis in a series of 36 cases of SMZL. VL+/VL-. Thirty-six patients were diagnosed as having SMZL on the basis of spleen histology and peripheral blood morphological and immunophenotypical evaluation. Twenty cases were classified as SMZL and 16 as Splenic lymphoma with villous lymphocytes. The two series showed similar clinical and laboratory features and differed only in the presence of circulating villous lymphocytes. Bone marrow trephine biopsy was performed with a Jamshidi needle and specimens were fixed in 10% buffered formalin. Bone marrow imprints for cytological evaluation were available in all cases. A small B-cell, mature appearing bone marrow infiltration was detected in all patients. An intrasinusoidal (IS) component was present alone or along with other patterns, mostly nodular (N) or interstitial (I), in all cases. As a whole, 11/36 (30%) cases showed an isolated IS infiltration and 13/36 (36%) IS+N, 10/36 (28%) IS+I, and only 2/36 (6%) IS+N+I. When a nodular pattern was present the nodules were never in the paratrabecular zone. The infiltrate was subtle (less than 20% of the bone marrow cellularity) in up to one fifth of cases. Moreover, when it was particularly scarce and exclusively IS it was barely detectable on H-E stains but easily recognizable when highlighted by CD20 immunostaining. The presence or absence of circulating villous lymphocytes did not influence the bone marrow infiltration pattern. Our experience indicates that bone marrow is always involved in SMZL patients. An IS component is invariably observed alone, or along with a nodular or interstitial component. As a small B-cell IS bone marrow infiltration has not been described in other lymphoma histotypes, this finding could be considered as a valuable and reliable diagnostic hallmark of SMZL thus avoiding an unnecessary diagnostic splenectomy. In our opinion, bone marrow biopsy should be a mandatory procedure in the differential diagnostic process of patients presenting splenomegaly.

**PO038**

**DIRECTIONAL POWER DOPPLER IMAGING FOR THE STUDY OF THE VASCULAR ARCHITECTURE OF ENLARGED SUPERFICIAL LYMPH NODES: WORK IN PROGRESS**

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Some interesting findings have previously been reported on the use of color Doppler ultrasound (US) examination for the differential diagnosis of peripheral enlarged lymphnodes. Directional power Doppler US imaging is a new technology that couples the ability to visualize the direction of blood flow in the vessels with better sensitivity even for very low flow velocities. This allows optimal visualization of the vascular architecture of enlarged superficial lymph nodes. The aim of our work was to evaluate this new US technology in the differential diagnosis of benign vs. malignant adenopathies. To this purpose, 50 patients (20 male, 30 female, age 18-75 yrs.), referred for a superficial adenopathy, underwent directional Power Doppler US imaging using a high frequency linear probe (Technos MP, Esaote, Spa, Milano). The vascular architecture of lymphnodes was studied in respect to vessel distribution and presence of hylar vascular pole. The vascular pattern was defined as regular (tree-shaped vascular distribution with vascular hylar pole) or irregular (irregular and peripheral distribution of the vessels, absence of the hylar vascular pole). All patients underwent subsequently surgical biopsy, core needle biopsy or fine needle aspiration for cytology. Twenty-seven patients had a neoplastic adenopathy (16 metastases and 11 lymphomas) and 23 patients had benign enlarged lymphnodes (6 cases of EBV infection, 3 cases of toxoplasmosis and 14 cases of aspecific reactive adenopathy). All patients with neoplastic adenopathies showed irregular vascular pattern at the Power Doppler US study. A case only of Hodgkin disease (nodular sclerosis) showed an intermediate vascular pattern, with vessel compression and distortion but presence of hylar vascular pole. Conversely, all patients with benign adenopathies showed regular vascular pattern at the US examination. Our preliminary findings suggest that the study of the vascular architecture using directional power Doppler study may substantially contribute to the differential diagnosis of the peripheral adenopathies. Moreover, the examination is noninvasive and relatively quick and simple.

**PO038**

**PRIMARY SALIVARY GLAND MALT LYMPHOMA: REPORT OF 31 CASES**

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Background: the majority of primary salivary gland lymphomas are classifiable as MALT. Although salivary glands represent one of the main sites involved by non-gastrointestinal MALT lymphomas, primary salivary glands MALT lymphomas (PSGML) are rare (<1% of all NHL), and data about their clinical behaviour quite limited. Moreover, the frequent association with autoimmune disease and, in particular, with Sjoegren Syn-
drome (SS) or HCV infection makes their pathogenesis intriguing. Aims: to analyze the clinical features at presentation, in particular the association with SS and HCV, and the outcomes in a large series of salivary gland MALT lymphoma. Methods: 31 cases of PSGML seen in our Institutions between 1989 and March 2003 were retrospectively evaluated. The diagnosis was made in all patients by histologic and immunohistologic analysis of biopsy or surgical specimens and was based on the REAL/WHO classifications. Staging procedure included routine lab, neck and abdominal ultrasound, CAT-scan and bone marrow biopsy (29 cases). Serology for HCV was tested in 24 patients. Results: they were 5 males and 26 females (M/F=0.2), aged 21 to 81 years (median 62). In 21 patients (67.7%), a single salivary gland was involved at diagnosis (parotid in 16, submandibular in 3 and minor salivary glands in 2); in 10 multiple salivary glands involvement was present. Other MALT organs (stomach and lacrimary gland respectively) were involved in only 2 cases. Bone marrow infiltration was documented in 2 patients. Ann Arbor stage was IE in 15 (48.4%), IIE in 3 (9.6%) and IV in 13 patients (42%). A history of SS was present in 13 cases, of scleroderma in 1; HCV infection was documented in 7. Overall, PSGML were associated with SS or other autoimmune diseases or HCV infection in 21/31 cases (67.7%); in no case HCV infection coexisted with autoimmune diseases. Treatment was mainly local: surgery in 15 patients (plus radiotherapy and/or chemotherapy in 8), radiotherapy alone in 3; 9 received chemotherapy (plus local radiotherapy in 2), 1 α-interferon; 3 were not treated. Overall, CR was achieved in 19/31 (61%), PR in 7/31 (22.5%). So far, disease progressed or relapsed in 12 patients, in 4 at lymphnodes, in 3 at distant extranodal sites (skin, lung, stomach). Histologic transformation in large B cell lymphoma occurred in 4/31 cases (13%), after 35 to 110 months. So far, 5 patients died, 3 of lymphoma, all after histological transformation, 2 of unrelated causes. The 5 years OS and PFS were 85±8% and 61±10% respectively. OS was not influenced by recurrence/progression. The only prognostic factors influencing OS were lymphnodal involvement and histologic transformation. Conclusions: the analysis of our series, which is one of the largest so far reported of PSGML, confirms that PSGML is an indolent disease, occurring preferentially in elderly women, which can be mostly managed with local treatment. Lymphnodal involvement and histologic transformation appear the only adverse prognostic factors. The frequent association with either SS or HCV infection strengthen the pathogenetic correlations between MALT lymphomas and chronic infections or autoimmune diseases.

PO040 REAL-TIME QUANTITATIVE PCR FOR MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE AFTER CHOP AND RITUXIMAB IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA PATIENTS

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The use of real-time quantitative polymerase chain reaction (RQ-PCR) has been proposed as a valuable tool to detect the BCL-2/IgH chimeric gene in follicular non-Hodgkin’s Lymphoma (FL-NHL) patients at diagnosis as well as to monitor minimal residual disease after different treatment modalities. We performed RQ-PCR analysis in 62 previously untreated FL-NHL patients at diagnosis as well as after the sequential administration of CHOP chemotherapy and Rituximab (Rambaldi et al. Blood, 2002, 99:856–62). The assay we applied (Ladetto et al.: Exp. Hematology, 2001, 29:183–93) allows the detection of 1 BCL2/IgH positive cell in about 105 normal cells. At diagnosis, the amount of neoplastic cells we detected in the bone marrow (BM, N = 52) and peripheral blood (PB, N = 40) was very heterogeneous ranging from cases with only negligible amount of tumor cells (1 Bcl-2/IgH positive cell in 105 normal cells) to patients showing a huge neoplastic infiltration (1 Bcl-2/IgH positive cell in 10 normal cells). When BM and PB from the same patient were analyzed simultaneously (N=30) the level of BM and PB infiltration was very similar in most cases. After six cycles of CHOP chemotherapy, the decrease of detectable Bcl-2/IgH positive cells remained in most cases within one log difference so that a molecular negativization could be achieved by CHOP chemotherapy only in those patients showing a BM infiltration of 10−4 or 10−5. A sequential Rituximab treatment (375 mg/m2 weekly×4) was given to 32 patients and minimal residual disease was evaluated 12, 28 and 44 weeks after baseline. At the first scheduled molecular follow-up (+12 weeks), the MRD evaluation performed both in the BM and PB showed that in patients with a high tumor load (10−2/10−4 neoplastic cells) at baseline, Rituximab treatment was followed by a median 3 logs reduction of the neoplastic infiltration. At the last molecular follow-up (+44 weeks), 68% of the patients remained PCR negative either in BM or PB. This study illustrates the great heterogeneity of neoplastic BCL2/IgH positive cells detectable in FL-NHL patients at diagnosis and underlines the different ability of CHOP chemotherapy and Rituximab in reducing the tumor load in the BM and PB.
PO041
HIGH INCIDENCE OF HEPATITIS B VIRUS REACTIVATION IN INDOMENT NON-HODGKIN’S LYMPHOMAS TREATED WITH FLUDARABINE-BASED REGIMEN: THE PROGNOSTIC ROLE OF VIRAL ACQUIRED GENOMIC MUTATIONS

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Hepatitis B virus infection is highly frequent in Asia, Africa, Latin America, and Southern Europe, where the percentage of HBsAg positive carriers in the general population ranges from 2 to 20%. Therefore it is not uncommon, in these countries, that a HBV carrier is a candidate for antineoplastic chemotherapy. It has recently shown that patients with non Hodkgin’s lymphoma (NHL) have the highest frequency of HBV reactivation, most likely for the high immunosuppressive steroid-based chemotherapy regimens used. At our knowledge, there are no data regarding acute hepatitis due to viral infection in patients with NHL treated by regimens containing fludarabine. In the present study, we evaluated 40 consecutive patients who underwent fludarabine-based induction chemotherapy for indolent NHLs, and investigated for incidence, risk factors, etiology, morbidity and mortality for acute liver damage related to hepatitis viral flare-up. Pretreatment assessment of viral serological status showed that, on the whole, 12 patients had a contact with HBV, and other 6 with HCV. Of the former group, 4 patients were HBsAg positive in a status of healthy carrier with undetectable serum HBV DNA sequences, other 2 were HBAb-positive only, and the remaining 6 had protective antibodies against HBsAg. After completion of therapy, HBV reactivation with acute liver damage was diagnosed in all the 4 HBsAg-positive patients and in 1 of the 2 HBcAb-positive patients, whereas neither the 6 HBsAb-positive patients, the 6 HCV-positive and the remaining 22 seronegative patients had acute hepatitis flare-up. Normalization of liver function tests and serum HBV-DNA disappearance were rapidly induced by lamivudine at daily dose of 100mg in 2 patients, while the remaining 3 patients had a severe and prolonged hepatitis who proved low responsiveness to the antiviral treatment. Interestingly, the severity of liver injury and the absence of response to lamivudine treatment correlate with the rate of HBV genome mutations of the strains isolated from patients at hepatitis flare-up. Indeed, we found that deviations from the closest sequences was 1.0% and 1.1% for the isolates from the 2 patients who had a rapid recovery of liver function tests, while was 1.5%, 1.8%, and 1.7% in the other 3 patients. The differences between viral strains isolated from the two groups of patients, was observed also at the polypeptide level. Indeed, the two subgroup of patients had a mean rate of amino acid substitutions respect to the closest sequences at the HBV polymerase open reading frame (ORF) and at HBs ORF of 2.7% vs 4.0% and of 1.25% vs 3.2%, respectively. In conclusion, we provide evidence that, in NHL patients, fludarabine based chemotherapy frequently induces reactivation of HBV latent viral replication, leading to clinical severe hepatitis. The observed high rate of genome mutations and amino acid substitutions at critical conserved domains of HBV proteins seems to play an important pathogenetic role for the viral reactivation, for the severity of clinical picture. Our results also indicate that HBV prophylaxis is required in NHL patients who are candidate for fludarabine-based chemotherapy.

PO042
CLINICAL RESOLUTION OF VANISHING BILE DUCT SYNDROME ASSOCIATED WITH HODGKIN’S LYMPHOMA: REPORT OF A CASE

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Development of cholestasis and jaundice in patients with Hodgkin’s lymphoma (HL) may be due to extrahepatic obstruction, lymphomatous infiltration, drug toxicity, hemolysis and post-transfusion hepatitis. The occurrence of vanishing bile duct syndrome (VBDS), with perportal fibrosis, ductular proliferation and disappearance of bile ducts in the portal spaces is a rare event which has been previously reported in sixteen cases only. In all the reported cases VBDS had a progressive course, leading to irreversible liver damage. The ursodeoxycholic acid (UDCA) is an effective treatment for intrahepatic cholestatic jaundice, that has never been tested in VBDS. A 42 year old Caucasian woman with a two-month history of recurrent episodes of jaundice, fever and weight loss attributed to cholangitis, was referred to our centre. A diagnosis of HL of nodular sclerosing type, stage IIB X (mediastinal bulky) was made. A liver biopsy excluded liver involvement by lymphoma and demonstrated VBDS. Oral 1350 mg/day ursodeoxycholic acid, 50 mg prednisone and full dose chemotherapy (ABVD) were started. The patient had a prompt response to chemotherapy and her cholestatic jaundice slowly improved. Figure 1 shows the time course of bilirubin, alanine aminotransferase, and alkaline phosphatase in relation to the treatment. She completed 6 ABVD cycles without reduction or delays fol-
lowed by consolidation radiotherapy in September 2002. Since then, she has been free of disease recurrence, without signs or symptoms of cholestasis. She is now continuing on 900 mg/day oral UDCA: a liver biopsy has been proposed to the patient, who momentarily refuses it. This is the first case showing that early specific treatment with high dose UDCA plus prednisone can reverse the biochemical and clinical features of the rarely occurring HL-associated VBDS and permit full chemotherapy course. We suggest that liver biopsy should be performed as early as possible whenever non-obstructive cholestasis develops in a patient with HL, so that VBDS may be diagnosed in an early stage and treated using high dose prolonged UDCA therapy combined with tapering doses of prednisone.

PO043
EFFECTS OF ANTHRACYCLINE-BASED CHEMOTHERAPY ON TOTAL PLASMA ANTIOXIDANT CAPACITY IN LYMPHOMA PATIENTS AND ITS MODULATION BY DEXRAZOXANE

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The clinical efficacy of anthracycline antineoplastic agents is limited by a high incidence of severe and usually irreversible cardiac toxicity; the cause of which remains controversial. Dextrazoxane clorhydrate (Cardoxane-→), a synthetic bisdiketopiperazine two-ringed compound which hydrolyzes to an EDTA analog, is thought to reduce cardiac toxicity by binding to free and bound iron, thus reducing the formation of anthracycline-iron complexes and the generation of free radicals which are toxic to cardiac tissue. Aim of the present study was to assess the effect of epirubicin-based chemotherapy on plasma free radical antioxid-ant capacity of patients with Non-Hodgkin Lymphoma (NHL), and the effect of supplementation with dextrazoxane on plasma antioxidant status. Methods. 14 untreated patients 60 years of age with newly-diagnosed aggressive NHL and treated with ProMECECytaBOM, were selected for the study. Other inclusion criteria were: performance status 0-3 (ECOG), normal cardiac function (EF 50%). The protocol was approved by the Ethical Committee of our Institution and fully informed consent was obtained from each subject entering the study. The patients were randomly allocated to receive or not dextrazoxane clorhydrate (40 mg/m²) after epirubicin infusion. Periferal blood samples in EDTA were done at baseline, after epirubicin infusion, and one hour later. Oxidative stress was evaluated by measuring at the beginning and at the end of the treatment plasma malondialdehyde (MDA) and plasma antioxidant capacity as its ability to antagonize the oxidation of α-keto-γ-methiolbutyric acid by hydroxyl radicals. The results are expressed as Total Oxyradical Scavenging Capacity (TOSC) units Results. Two hours after epirubicin infusion, plasma MDA was significantly increased (2.8±0.3 vs. 5.8±1.1 mol/L, p<0.001) and plasma TOSC against hydroxyl radicals significantly reduced (33.2±8.4 vs. 12.5±4.3 U, p<0.0001) in the whole study population. Of interest, patients who underwent supplementation with dextrazoxane clorhydrate exhibited a significant increase in plasma anti-hydroxyl radicals antioxidant capacity, respect to epirubicin infusion (11.9±5.1 vs. 27.4±6.8 U, p<0.001), as well as respect to those patients who had placebo (12.8±3.7 vs. 18.5±4.2 U, n.s.). Conclusion. These results are in accordance with previous studies showing the formation of oxidants with the use of anthracycline, and suggest that dextrazoxane may protect against anthracyclines-induced damage to myocytes by preventing iron-based oxygen radical damage.

PO044
MILD ACTIVE CHRONIC HEPATITIS AS A PRESENTING PARANEOPLASTIC MANIFESTATION OF HODGKIN’S DISEASE

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Liver is involved in about 5-8% of newly diagnosed Hodgkin’s disease (HD) cases and is often associated with intrahepatic cholestasis. Acute hepatic failure secondary to involvement by hematologic malignancies at presentation is an uncommon condition and usually has a very poor prognosis. We described a patient with Hodgkin’s disease (stage IIIA) with hepatitis without evidence of liver lymphoma infiltration that favourably
evolved after chemotherapy. Case report: a 31-year-old man presented a lymphadenopathy in the right inguinal region due to classic Hodgkin’s disease (mixed cellularity) as revealed by histological analysis of a surgery biopsy. Initial investigation demonstrated hepatocellular injury as revealed by the increase of aspartate transaminase (AST), 723 IU/L (n.r.< 37 IU/L); alanine transaminase (ALT), 1410 IU/L (n.r.< 40 IU/L) lactate dehydrogenase LDH, 893 IU/L (n. r. 230-460; U/L) as well as mild cholestasis with bilirubin, 1.83 mg/dL γ-glutamyl transpeptidase, 124 IU/L (n.r.< 50 IU/L) and normal alkaline phosphatase. No jaundice or liver functional failure was present. He had no history of alcohol or drug abuse and no family history for liver disorders. Serologic tests for hepatitis A, B and C, cytomegalovirus, Epstein-Barr virus, Parvovirus, human immunodeficiency Virus 1 and 2 were negative. Serum mitochondrial (AMA), smooth muscle (ASM A), microsomial liver (LKM) and nuclear (ANA) antibodies were absent excluding autoimmune syndrome. A computed tomography scan of the chest, abdomen and pelvis was remarkable for axillary and multiple abdominal lymphadenopathy (diameter up to 6 cm). Moreover, ultrasonic abdominal examination demonstrated a slight enlarged but otherwise normal liver, normal biliary tree with no evidence of biliary obstruction. Splenomegaly with a 15 cm diameter was also present. FDG-PET imaging was able to detect abdominal lymphadenopathy but not liver, spleen and axillary regions involvement. Bone marrow biopsy showed no lymphoma infiltration. A percutaneous liver biopsy revealed a mild active chronic hepatitis with no evidence of infiltration by Hodgkin’s disease. A chemotherapy regimen according to the ABVD schedule (doxorubicin, bleomycin, vinblastine, dacarbazine) was started. After two courses of chemotherapy a significant improvement of liver functions was noted obtaining a complete normalization of the levels of serum transaminases and of cholestatic parameters. Paraneoplastic syndromes complicating a Hodgkin’s disease have been described especially with neurologic, renal or cutaneous involvement. Review of the literature revealed only very few cases of idiopathic cholestatic jaundice or fulminant hepatic failure without a demonstrable cause suggesting a paraneoplastic etiology. In this report we described a rare case of mild active chronic hepatitis in Hodgkin’s disease without evidence of lymphoma infiltration that should be considered as a paraneoplastic syndrome; this condition may be reversible and may respond to conventional chemotherapy. Thus, paraneoplastic hepatitis would be considered in the differential diagnosis of liver injury at the presentation of Hodgkin’s disease.

PO045
CD30 POSITIVE PARAGANGLIOMA MIMICKING HODGKIN’S DISEASE RELAPSE
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Diagnosis of relapsing Hodgkin’s disease (HD) usually relies on histological examination of surgical biopsies from clinically suspect recurrence sites. Cytological evaluation of fine needle aspirates (FNAB), along with detection of CD30+ tumor cells by flow cytometry and/or immunocytochemistry, has been recently proposed as a reliable alternative to surgical biopsy. The typical cytologic appearance of Hodgkin and Reed-Sternberg (H/RS) cells and the infrequent expression of CD30 by other tumor cell types, represent the diagnostic standpoints of this approach. In September 1988 a 15 year old girl was referred at our institution due to a large inguinal lymphadenopathies. A diagnosis of stage IIA nodular sclerosis HD was made upon incisional biopsy followed by staging laparatomy. Treatment with inverted Y radiotherapy resulted in a complete response (CR) which was maintained until December 1998 when the patient presented with a left axillary lymphadenopathy. A further biopsy was histologically consistent with HD relapse. The patient obtained a second CR following 6 courses of ABVD and no disease recurrence was observed during the subsequent five years follow-up. In May 2003 the patient developed a left cervical mass of 55×50 mm. Clinical examination, neck ultrasonography and imaging identified a large 32×22 mm mass and two additional 20×20 mm masses respectively located at the jugulo-carotid and jugulo-digastric junctions, compatible with pathologic lymph nodes. The overall picture was therefore strongly suggestive of HD relapse. A new excisional biopsy of the larger mass was performed for diagnostic purposes and part of this tissue was processed for immunomagnetic isolation of putative H/RS cells by means of magnetically labelled CD30 M ultisort M icrobeads using the VarioMACS system (Miltenyi Biotec, Auburn, CA, USA). CD30+ enriched tumor cells were cytologically assessed by hematoxylin/eosine and immunocytochemical staining with anti-CD30 mAbs. Large multinuclear and mononuclear cells expressing CD30 and resembling H/RS cells, were readily detected following such procedures. Moreover, RT-PCR amplification of mRNA obtained from the positive and negative cell fractions yielded the expected 860 bp CD30-specific
amplicon on mRNA from the CD30+ enriched cell population, but not from CD30− cell fraction. Despite all the above results, however, the final histological diagnosis of the excised mass was of parasympathetic paraganglioma of the carotid body with chromogranin A-positive chief cells and S100-positive sustentacular cells. A high mitotic rate of tumor cells was also evidenced by Ki-67 assessment. Additional staining of histological sections of the excised tumor revealed a consistent expression of the CD30 antigen by chief cells. Since no published evidence of CD30 expression on paraganglioma cells was previously described, 10 additional paraganglioma specimens were collected from different institutions and investigated for CD30 immunostaining. The results showed that in all analyzed cases, the great majority of endocrine tumor cells were CD30 positive, displaying either a paranuclear dot-like or a diffuse cytoplasmic immunoreactivity. This study emphasizes the risk of misdiagnosing HD relapses on merely cytologic and even immunocytochemical analysis of FNAB, and reiterate the absolute need for surgical biopsy of recurrence sites. Standard histological assessment of neoplastic tissues remains mandatory to avoid inappropriate treatment even if clinical history and imaging are strongly suggestive for a disease relapse. More importantly, we provide the first report documenting expression of CD30 antigen in paraganglioma tumor cells.

P0046
PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA IN IMMUNOCOMPETENT ADULTS: ANALYSIS OF A RETROSPECTIVE SERIES OF PATIENTS TREATED WITH IDARUBICIN-CONTAINING REGIMEN AND RADIOTHERAPY
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Introduction: Primary central nervous system lymphoma (PCNSL) is an aggressive and very rare brain tumor. However, in the last decades its incidence is increasing representing 0.85-6% of all cerebral tumours and 0.9-4.2% of all the extranodal malignant lymphomas in particular in HIV-infected subjects (6%) and in pts receiving organ transplantation and immunosuppressive therapy (22%). In this study, we report the analysis of 9 consecutive pts treated with protocols containing standard doses of chemotherapy regimens including idarubicin instead of doxorubicin because its ability to cross beyond the blood-brain barrier. Design and Methods: Between December 1993 and May 2002, 9 consecutive PCNSL were diagnosed and treated at our Institution with combined modality treatment (CMT) (chemotherapy plus standard whole brain RT). Median age: 51.8 years (range: 23 to 69). All pts had aggressive B-phenotype histology (7 DLBC, 1 immunoblastic, and 1 Burkitt-like NHL), diagnosed by stereotactic biopsy. The treatments was CIOP chemotherapy, a CHOP-like regimen including idarubicin at 12 mg/m² instead of standard dose doxorubicin (n=7) and ProMICE-CytaBOM chemotherapy (n=2) differing from standard schedule because of idarubicin given at 8 mg/m² on d1. All pts received additional concomitant intrathecal therapy with methotrexate 12 mg, aracyn 30 mg and prednisone 20 mg. Results: Overall response rate was 77%. CR 44% (n=4) and PR 33% (n=3). One patient (11%) is alive and disease-free at 5 year. The median survival time was 8 months. All Pts achieving PR died of progressive disease with a median time to progression of 5 months. Three out of 4 Pts in CR relapsed at +5, +8 and +37 months, and all of them died of progressive disease. Regarding the toxicities (scored according to WHO-criteria), no Grade 4 toxicities were observed. G3 neutropenia occurred in 6/9 (66%); anemia G1 in 5/9 (55%); grade 2 neuropathy was registered in all pts; grade 2 mucositis in 2/9 (22%) pts. No cardiotoxic effects have been registered. Conclusions: The optimal treatment of pts with PCNSL remains still to be defined; however, with conventional standard regimens the prognosis remain still poor. The our experience with idarubicin-containing regimens combined with WB-RT did not improve the outcome of patients with PCNSL. New strategies should be investigated to increase the cure rate of these patients including high dose therapy and stem-cell rescue.

P0047
RISK FACTORS, TREATMENT, AND OUTCOME OF CENTRAL NERVOUS SYSTEM RECURRENCE IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA
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Central nervous system (CNS) recurrence was evaluated in terms of incidence, risk factors, and outcome in a consecutive cohort of 158 patients (pts) with newly diagnosed intermediate-aggressive non-Hodgkin’s lymphoma (NHL), excluding lymphoblastic and Burkitt’s lymphoma. No prophylactic intrathecal treatment was administered at diagnosis. Thirteen pts (8%) developed CNS recurrence after a median of 6 months from diagnosis (range, 1 to 28 months). Eleven pts had diffuse
large-cell, and two follicular lymphoma. At diagnosis, seven pts had stage IV disease, 7 abdomen bulky disease, and 8 IPI > 2. Bone marrow was involved in only one case. In five cases, CNS involvement was the only site of relapse. Personality change and cranial nerve palsies were the most common presenting symptoms, followed by obtundation, headache, and peripheral sensory or motor symptoms. CNS lymphoma recurrence was documented by cerebrospinal fluid cytology in 3 pts, by radiographic findings in 3 pts, and by both procedures in the remaining pts. In univariate analysis, the only factor significantly associated with a greater likelihood of CNS recurrence was LDH (p=0.003) at diagnosis. Ten pts received systemic chemotherapy combined with intrathecal treatment. The chemotherapy was cytarabine-based in 7 pts, and consisted of high-dose of methotrexate in 3 pts. Intrathecal therapy consisted of cytarabine, metotrexate, and hydrocortisone. Three pts received intrathecal chemotherapy plus whole-brain radiotherapy (40 Gy). Objective response of the CNS disease was observed in 3 pts, and 2 pts obtained a durable remission. Two pts are still alive, in CNS remission at 33 and 10 months, respectively, after diagnosis of recurrence. Median survival of the whole series is only 2 months confirming the poor prognosis after CNS recurrence and stressing the need to develop new treatment strategies. New biological markers and clinical scores are necessary to better identify patients at an high risk of CNS recurrence.

**PO048**

DISAPPEARANCE OF TWO IgGk AND IGGA MONOCLONAL GAMMOPATHIES IN HHV8+ CASTLEMAN’S DISEASE AFTER SPLENECTOMY

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Multicentric Castleman’s disease (MCD) is a systemic lymphoproliferative disorder characterized by angiofollicular lymphoid proliferation causing fever, lymphadenopathy, hepatosplenomegaly, rash. This uncommon non-neoplastic disease is associated with an increased risk of Kaposis sarcoma and lymphoid cancer. In addition, both Kaposis sarcoma and MCD are characterized by vascular hyperplasia, dysregulation of the immune system Human Herpes Virus 8 has been demonstrated in tissues obtained from patients with Kaposis sarcoma (KS) and multicentric Castleman’s disease (MCD) and in primary effusion lymphoma (PEL) cells or body cavity based lymphoma cells. Epidemiological studies provide a picture of the patterns of HHV8 infection, but we do not known precisely how the virus is transmitted. The pathogenetic effects of HHV8 are better known. Polymerase chain reaction (PCR) analysis has indicated that HHV8 can be detected in 95% of KS and MCD lesions. HHV8 may infect IgM+ naive B cells and drive these cells to differentiate into plasmablasts without undergoing germinal center reaction. Interleukin-6 (IL-6) is produced in KSHV/HHV8+ cells from MCD lesions, and may play a role in the plasmacytic differentiation of the cells Viral IL-6, however, blocks Interferon signalling. The good treatment of Castelman’s disease and/or HHV8 infection is not standardized, for this reason we report a case patient. We recently cared for a patient who had HHV8 infection associated with multicentric Castleman’s disease with rapidly increase of spleenomegaly. In may 2000, a 36-year-old man began to notice for control check up the bioumoral data demonstrated a monoclonal gammapathy with uncertain significance. The clinical observations demonstrated a rapidly increase of spleenomegaly. Computed tomography (01/31/2001) demonstrated the presence of lesions with difficult to explain. The increase significantly of two monoclonal component IgGk 2.8 g/dL and 10.9 g/dL, the bone marrow biopsy normal, rx, tac total body no lymphadenopathies we hypothesized a suspected non-Hodgkin’s lymphoma as lymphoplasmocytoid lymphoma and consider the splenectomy. The diagnosis is Castelman’s disease, the research of HHV8 antibody title demonstrated the presence of the virus. No therapy was started and after 2 years the patients have a good quality of life and the complete disappearance of two gammapathies has been demonstrated.

**PO049**

PRIMARY SPLENIC LYMPHOMA. REPORT OF 15 CASES WITH EMPHASIS OF ULTRASONICALLY-GUIDED TISSUE-CORE NEEDLE BIOPSY IN THE DIAGNOSIS AND MANAGEMENT

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Malignant lymphoma seldom presents as a primary neoplasm of the spleen. Das Gupta et al, proposed strict criteria for establishing a diagnosis of PSL: it can be made when the disease is confined to the spleen, or ilar lymphonodes. We report here 17 cases of PSL defined by Das Gupta’s criteria and we emphasize the usefulness and safety of percutaneous ultrasonically guide fine-needle biopsy (UFG-FNB) in the diagnosis and management of this subset of patients. All the 17 patients were previously undiagnosed and showed suspicion of splenic disease at clinical or imaging examination. Suspicion of splenic disease was defined as presence of splenic focal lesions or splenomegaly without focal...
lesions. All the patients underwent bone-marrow biopsy, laboratory tests and imaging studies, prior to other invasive procedures. Patients with focal splenic lesions underwent UG-FNB of the splenic lesions using a 21-gauge biopsy needle, while patients with splenomegaly alone underwent splenectomy. Five patients (29.4%) showed splenomegaly without focal lesions, twelve patients (64.7%) had splenic focal lesions, three of them (25%) had splenomegaly, while in nine patients the spleen was not palpable. Percutaneous core biopsy allowed a refined diagnosis in 10/12 cases (83.3%). The histologic diagnosis were: 8 diffuse large cell lymphoma, 2 type 1 follicular lymphoma (FL). In the remaining 2 cases the diagnosis could not be established with needle biopsy alone and the patients underwent diagnostic splenectomy that showed type 1 FL and marginal zone lymphoma (MZL). The five patients with splenomegaly without focal lesions underwent splenectomy and the histopathologic examination showed MZL in 4 cases and type 2 FL in 1 case. It must be emphasized that the diagnosis of PSL is currently made with splenectomy. From our experience in this study UG-FNB provides sufficient information for the diagnosis of PSL, in patients with splenic focal lesions, and subsequent therapeutic decision to treat most of these cases avoiding further surgical procedures.

**Acute Myeloid Leukemias and Myelodysplastic Syndromes I**

**PO050**

**The Quantitative Assessment of WT1 Is a Potent Tool for Predicting Relapse Feasible in Peripheral Blood of Acute Leukemia Patients**

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Detection and monitoring of minimal residual disease (MRD) have been shown to have prognostic significance in the management of leukemias and may provide the basis for a patient tailored therapy. RT-PCR protocols aimed at detection of the most common fusion transcripts present in leukemias are the most sensitive techniques. However, they are suitable only in about 40% of acute myeloid leukemias (AML) and in 30-35% of acute lymphoid leukemias (ALL). We have previously demonstrated that WT1 is a universal leukemic marker useful for MRD detection in all the cases of AML and ALL patients. In order to determine the potential use of this marker in peripheral blood for early prediction of relapse, we performed a quantitative Real-Time PCR (TaqMan) assay in 42 patients (32 AML, and 10 ALL) at diagnosis and during follow-up (a median of 4 samples per patients were tested). All the patients were characterized at the cytogenetic and molecular levels for the presence of the most frequent genetic abnormalities. In addition we tested the expression level in 52 PB samples from healthy volunteers as controls. We found that the peripheral blood of healthy donors expressed very low levels of WT1 with the majority of samples which scored negative. The median values of the positive samples was 5 WT1 copies/10^4 ABL copies (range1-22). By contrast, in all the peripheral blood samples collected from leukemia patients at diagnosis the WT1 transcript was increased above the normal range (median value of WT1 copies/10^4 ABL copies:10244, range 758-86140 in AML and 1388, range 212-34707 in ALL). In all the patients who reached a complete remission after chemotherapy, WT1 returned within the normal range. In all the patients who persisted in CR, WT1 was persistently found within the normal range during follow-up. By contrast, all the cases (14 AML and 4 ALL) in which during follow-up the WT1 increased above the normal range in the PB subsequently relapsed after a period ranging from 1 to 4 months. In addition, in 7 AML cases with normal karyotype, the immunophenotypical analysis carried out in BM samples after chemotherapy was suggestive for CR, but WT1 tran-
script amount detected in PB persisted at levels above the normal range. All these patients relapsed during follow-up in a period ranging from 1 to 6 months. These data clearly demonstrate that WT1 is a very sensitive marker for early prediction of relapse in PB. So, the quantitative assessment of WT1 in PB samples, offers the possibility to monitor with high frequency all the cases of acute leukemias and to predict relapse same months before it becomes clinically evident.

PO051
GENTUZUMAB OZOGAMICIN ("MYLOTARG") FOR MOLECULAR RELAPSE OF ACUTE PROMYELOCYTIC LEUKEMIA
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CD33 is brightly expressed on acute promyelocytic leukemia (APL) cells and two studies have reported the efficacy of Mylotarg, a humanized anti-CD33 monoclonal antibody conjugated to calicheamicin, in APL untreated patients (84% of responses) or after retinoic acid (RA) and/or chemotherapy for induction of molecular remission (50% of responses). We report our experience on Mylotarg as a single agent for molecular remission (50% of responses). We report our experience on Mylotarg as a single agent for molecular remission (50% of responses). We report our experience on Mylotarg as a single agent for molecular remission (50% of responses).

The remaining 10 patients are in MR after mylotarg cycles until negativization of the molecular remission. Of these, two received again mylotarg at the same dosage; one responded again undergoing MR and one is under evaluation. The two other patients had relapsed after BMT; both received donor lymphocyte infusion and underwent new molecular remission. Thirteen patients (93%) achieved molecular remission as documented by the RT-PCR tests carried out after 2 cycles. The other patient had a morphologic relapse after two doses. The most common adverse reactions after completion of the infusion included low-grade fever, mild nausea, headache and polyuria in 1 patient. Four episodes of neutropenic fever of unknown origin (FUO) were observed. Mild neutropenia was observed after the first cycle, with a median duration of 8 days and after second and third cycle with median duration of 10-12 days. Thrombocytopenia (grade 1) lasting for 3-5 days occurred after second and third doses. Five patients developed asymptomatic increase of transaminase during the first week of treatment. Only one patient with concomitant GVHD after allogeneic BMT had hepatic toxicity (grade 2-3) without veno-occlusive disease. Four patients relapsed at molecular level after 5, 6, 8 and 13 months of mylotarg-induced MR. Of these, two received again mylotarg at the same dosage; one responded again undergoing MR and one is under evaluation. The two other patients had relapsed after BMT; both received donor lymphocyte infusion and underwent new molecular remission. The remaining 10 patients are in MR after mylotarg after a median follow up of 5+ (range 2.5 -23) months. Our results indicate that Mylotarg has activity as a single agent against minimal residual disease in advanced APL. A higher patient number with prolonged follow-up needs to be evaluated.

PO052
RAPID DETECTION OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS BY DENATURATING-HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY
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FLT3 is the most commonly mutated gene in human acute myeloid leukemia (AML), and has been implicated in its pathogenesis. Since screening of FLT3 in AML patients by PCR followed by gel electrophoresis is time-consuming and fails to detect some very small ITDs, we set up a method for screening of FLT3 receptor mutations with PCR plus denaturing high-performance liquid chromatography (D-HPLC). Total mRNAs extracted from 34 AML patients were first analysed for the presence of JM length-mutations and TKD point mutations by conventional method involving PCR amplifications, restriction enzyme digestion and agarose-gel electrophoresis (PCR-RE-AGE). Subsequently, the same

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patients panel was analysed by D-HPLC, using specifically designed primers and optimised running temperatures for the length and point mutations analysis. Thirty-four patients were analyzed by PCR-RE-AGE: nine were found to be positive for known FLT3 mutations (9/34 corresponding to 26.5%; length mutations plus point mutations). Most importantly, additional nucleotide changes were discovered; direct sequencing analysis identified nucleotide alterations in each of the D-HPLC-positive but not in any of the D-HPLC-negative samples, yielding a specificity and sensitivity of 100% for D-HPLC based screening of FLT3 receptor. This first description of mutational analysis by D-HPLC based method for detection of ITDs and TKDs mutations in FLT3 gene should be useful for studies involving precise genotype determination, which could be critical for selection of innovative AML therapies targeting the FLT3 gene. Moreover, this technique has been used for further identification of new point mutations occurring in the regulatory and catalytic domains of FLT3 protein, which are considered a hot-spot region for activating mutations in the class III RTKs subfamily.

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P0053
SECOND MALIGNANCY AFTER TREATMENT OF ADULT ACUTE MYELOID LEUKEMIA
GIMEMA

Purpose: To quantify the relative and absolute excess risks of second malignancies (SM) among patients affected by acute myeloid leukemia (AML) enrolled in the GIMEMA clinical trials. Design and Methods: A prospective non-concurrent analysis was performed on 3484 new AML patients consecutively enrolled in a multi-center setting in the GIMEMA clinical trials, between 1982 and 1999. Results: Among 2603 patients achieving complete remission (75%), 19 patients (0.7%) developed a SM, corresponding to 2.9 per 10,000 person-year incidence of SM. This incidence was lower than expected (SIR 0.69, 95% CI 0.41-1.07), when compared to that of Italian Cancer Registries. However, it resulted significantly higher in AML patients younger than 60 years (SIR 3.63, 95% CI 1.56-7.15), when stratifying patients according to age. The estimated cumulative incidence of a SM over 5 years was similar for patients older or younger than 60 years (3.9% and 4.7% respectively), while it was lower for SM patients with a previous acute promyelocytic leukemia (APL, 0.44%). A significant number of acute myelo-monocytic leukemia patients developed a SM (7/440 vs 12/2163; OR 2.90, CI 95% 1.03-7.95; p=0.03), which translated in a 1.44-fold increased risk, compared to the normal population. Conclusion: The incidence of SM in adult AML is lower than expected in the normal population. The stratification according to age showed that patients younger than 60 years and patients with acute myelo-monocytic leukemia had an increased risk of SM. On the contrary, the rarity of SM in APL patients suggests that treatments for APL do not have a role in the development of SM. Our data show that the cumulative risk of SM in adults cured for AML or APL is not clinically relevant.

P0054
FLUDARABINE AND CYTARABINE AS CONTINUOUS SEQUENTIAL INFUSION PLUS LIPOSOMAL DAURONUBICIN (DAUNOXOME) AS SALVAGE THERAPY FOR REFRACTORY OR RELAPSED PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Relapse represents the main adverse prognostic factor in patients with AML. In particular, early relapse and disease recurrence after autologous or allogeneic stem cell transplantation (SCT) as well as primary resistance to induction chemotherapy are associated with poor results in terms of long-term survival and cure. For these patients, new approaches are clearly needed. The combination of fludarabine (FAMP) and cytarabine (ARA-C) ± G-CSF has been proven effective in poor risk AML. Aiming to increase the synergistic activity of these two drugs, we developed a therapeutic program based on the combination of FAMP/ARA-C given as continuous sequential infusion associated with DAUNOXOME. The regimen consisted of a sequential infusion in which FAMP was administered at a loading dose of 10 mg/sqm on d 1, followed by ARA-C at 390 mg/sqm in 3 hrs on d 1; then both FAMP (25 mg/m² d 1,2) and ARA-C (1900 mg/m² d 1,2,3) were given as continuous infusion. Daunoxome (60 mg/m²) was added on d 2,3,4. A consolidation therapy, using the same schedule with only two doses of Daunoxome (d 2 and 3) was administered to responding patients. Then, patients who were eligible for a SCT underwent autologous or allogeneic transplant.
Inactivation of this gene is critical for cancer development. In immortalized cell lines which frequently lack DAP-kinase expression, the latter may be restored by treatment with demethylating agents, including 5-aza-2'-deoxycytidine restoring their sensitivity to induction of apoptosis by gamma-interferon. Using a methylation-specific PCR, we studied the methylhylation status of DAP-kinase in 160 bone marrow samples from adults with acute myeloid leukemias (AML) at the time of initial diagnosis. We observed hypermethylation of DAP-kinase in 27.5% (44/160) of AML specimens, and hypermethylation significantly correlated to loss of DAP-kinase expression studied by RT-PCR (p=0.018). Hypermethylation of DAP-kinase was significantly more frequent in AML secondary to therapy for other malignancies (s-AML, 14/29, 48.3%), as compared to de novo AML (30/131, 22.9%, p=0.01, OR: 3.1, 95% CI: 1.4-7.2). DAP-kinase hypermethylation in AML was associated to myelodysplastic changes in the bone marrow at the time of the initial diagnosis (p=0.002), but not to other patients' characteristics including age, gender, FAB subtype and cytogenetic risk group.

Gene silencing of tumor suppressor genes by methylation can contribute to cancer development. Death-associated protein kinase (DAP-kinase) is a pro-apoptotic calcium/calmodulin-regulated serine/threonine kinase with a multidomain structure that participates in several apoptotic pathways initiated by IFN-gamma, TNF-alpha, activated Fas, and detachment from extracellular matrix. DAP-kinase is abnormally methylated in a significant proportion of human tumors, providing evidence that inactivation of this gene is critical for cancer development. In immortalized cell lines which frequently lack DAP-kinase expression, the latter may be restored by treatment with demethylating agents, including 5-aza-2'-deoxycytidine restoring their sensitivity to induction of apoptosis by gamma-interferon. Using a methylation-specific PCR, we studied the methylhylation status of DAP-kinase in 160 bone marrow samples from adults with acute myeloid leukemias (AML) at the time of initial diagnosis. We observed hypermethylation of DAP-kinase in 27.5% (44/160) of AML specimens, and hypermethylation significantly correlated to loss of DAP-kinase expression studied by RT-PCR (p=0.018). Hypermethylation of DAP-kinase was significantly more frequent in AML secondary to therapy for other malignancies (s-AML, 14/29, 48.3%), as compared to de novo AML (30/131, 22.9%, p=0.01, OR: 3.1, 95% CI: 1.4-7.2). DAP-kinase hypermethylation in AML was associated to myelodysplastic changes in the bone marrow at the time of the initial diagnosis (p=0.002), but not to other patients' characteristics including age, gender, FAB subtype and cytogenetic risk group. We therefore studied the methylation status of DAP-kinase in patients with myelodysplastic syndromes. DAP-kinase hypermethylation was a frequent event in MDS, with 17 hypermethylated bone marrow samples of 35 MDS patients (48.6%). These data suggest that hypermethylation of DAP-kinase may be an early event in the malignant transformation of myeloid cells. Alteration in the apoptotic response due to the loss of DAP-kinase function may play a functional role in the transformation pathway to secondary leukemia via myelodysplasia. These data point to a role for the inclusion of demethylating agents into treatment protocols for acute myeloid leukemia and myelodysplastic syndromes, in particular for therapy-related forms.
Acute myeloid leukemias (AMLs) are a heterogeneous family of hematopoietic malignancies that share a high frequency and a high degree of Pgp-mediated multidrug resistance (MDR), one of the major causes of treatment failure. Induction treatment (FLAI), including 5 days administration of Idarubicin (IDA-10mg/m²/day), in combination with high-dose Arabinosyl Cytosine (HDAC 2 g/m²/day) and Fludarabine (FLUDA 25 mg/m²/day) was adopted as induction treatment of newly diagnosed patients with AML, except acute promyelocytic leukemia (APL). In our previous experience (Russo et al. Leuk Lymphoma, 2001), FLAI regimen showed to be highly effective (CR rate 72%), also in AML MDR-pos patients, with a low extra-hematological toxicity. In this prospective randomized trial, FLAI was compared with ICE (IDA 10mg/m²/day×3 days + AC 100mg/m²/day×10 days + VP16 100mg/m²/day×4 days) for induction of remission. Post-induction treatment included: HDAC (3g/m²/12h×6 days), MEC (Mitoxantrone 12 mg/m²/day×4 days), Etoposide 100 mg/m²/day×4 days, Cytarabine 1 g/m²/day×4 days) and allogeneic or autologous BMT. Over a period of 2 years, 122 patients were randomly assigned to FLAI (67) or ICE (55). The clinical and hematologic characteristics of the two patient population were not different. In the FLAI group, the complete remission (CR) rate was 72% after the first course and 76% after HDAC; in the ICE group, the CR rate was 50% and 63%, respectively (p<0.02). Interestingly, the CR rate of MDR-pos. and MDR-neg. patients treated with FLAI was similar (68% vs 65%), while the CR rate of MDR-pos. and MDR-neg. patients treated with ICE showed a significant trend in favour of MDR negative patients (20% vs 54%). The median time to recovery of neutrophils was seen an approximately equal rate of FUO, Gram negative/Gram positive bacteremias and systemic fungal infections. Infections and hemorrhages caused death during induction (DDI) in 3% of patients treated with FLAI and in 9% of patients treated with ICE. Non-hematological toxicity of FLAI was mild and significantly lower than ICE. In particular, in the FLAI arm, only 3/67 patients developed a grade 3 or 4 gastrointestinal toxicity, whereas in ICE arm 16/55 patients experienced this toxicity (p=0.0001). Other grade 3 or 4 toxicity (i.e. transaminasemia, cutaneous toxicity, renal or cardiac failure) were seen in 1/67 patients in FLAI arm versus 7/55 patients in ICE arm (p=0.02). In conclusion, these preliminary results strongly suggest that FLAI, as a single induction course, is an highly effective regimen with a limited non-hematologic toxicity. Furthermore, FLAI seems to be more effective than ICE in overcoming Pgp-mediated multidrug resistance (MDR).

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PO057
THE PRION-LIKE PROTEIN DOPPEL IS EXPRESSED IN BLAST CELLS OF ACUTE LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES

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The PRND gene, located on human chromosome 20p, 16 Kb downstream from PRNP, the prion protein gene, encodes for the Doppel protein (Dpl), that has many biochemical and structural properties in common with the prion protein. Dpl is about 125 amino acid residues long, it is characterized by an α-helical conformation, and it is presented on the cell surface by a glycosylphosphatidylinositol anchor. Whereas prion protein is ubiquitous, Dpl is expressed only in adult testis and does not seem to be required for prion disease progression. Its physiological role is unknown. Since preliminary studies have demonstrated a significant level of Dpl expression in several types of cancers of different histologic origin, we investigated the Dpl distribution in bone marrow cells from patients with acute leukemia (AL) and myelodysplastic syndrome (MDS), in order to evaluate its possible overexpression in these disorders. Molecular and immunocytochemical studies were carried out on bone marrow samples from 9 normal controls, from 14 patients with AL, 7 myeloid (AML) and 7 lymphoid (ALL), at the onset, and from 23 patients with MDS (10 RA, 5 RARS, 5 RAEB, 1 RAEB-t and 2 CMML) not previously treated. A goat polyclonal antibody raised against a peptide mapping near the aminoterminus of Dpl of human origin was used for immunocytochemistry and Western blotting. Dpl protein was detected in 6/7 cases of AML, in 5/7 cases of ALL and in 21/23 cases of MDS, whereas normal samples were negative or showed very weak expression in rare immature myeloid cells. Dpl was localized on the blast surface or, seldom, in a cytoplasmic perinuclear area. In AL and MDS the number of positive cells and intensity of staining were heterogeneous. Semi-quantitative RT-PCR demonstrated rather high mRNA levels in almost all AL and MDS cases, but barely detectable levels in normal bone marrow. These differences were likely not related to gene amplification. In AL and MDS there was no correlation between Dpl
expression levels and clinical or laboratory findings such as age, leukocyte count or karyotype. In conclusion, for the first time the expression of PRND has been demonstrated in human bone marrow cells. The molecular mechanism responsible for its overexpression in transformed cells is unknown; however, the differential expression of the Dpl protein in AL and MDS versus healthy subjects makes it a possible leukemia associated antigen with a potential attractive role as target for immunotherapy; on the other hand, Dpl could be used to quantitate minimal residual disease after treatment.

PO058
SURVIVIN EXPRESSION IN ACUTE LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES
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Survivin is an inhibitor of apoptosis of the IAP gene family, that at the beginning of mitosis associates with microtubules of the mitotic spindle. Present during embryonic and fetal development, it was undetectable in normal adult tissues, but overexpressed in various human neoplasias, acute leukemias included. Survivin expression in solid cancers correlated with unfavorable disease and shortened survival. However, no data have been reported on the potential role of this cell cycle-regulated anti-apoptotic pathway in myelodysplastic syndromes (MDS). We analyzed the expression of survivin in bone marrow cells from 44 patients with acute leukemia (AL), 20 myeloid (AML), 16 de novo and 4 secondary, and 24 lymphoid (ALL), 5 T and 19 of the B-lineage, at the onset, from 73 patients with MDS (27 RA, 17 RARS, 13 RAEB, 10 RAEB-t and 6 CMML) not previously treated, and from 22 non hemopathic subjects. Our aim was to evaluate whether abnormalities in its expression were associated with peculiar laboratory and clinical findings. Moreover, a possible correlation was investigated between survivin positivity and altered apoptosis level, as measured by TUNEL technique, or altered proliferative rate, as evaluated by MIB-1 immunostaining. Survivin was detected by an immunoalkaline phosphatase method (streptavidin-biotin complex) using a primary murine monoclonal antibody raised against human recombinant survivin (clone 8E2, NeoMarkers). In normal samples survivin was never detectable. In AL and MDS the number of positive cells and intensity of staining were heterogeneous. Survivin was localized in the cytoplasm of blasts in a para- or perinuclear area. It was detected in 15/20 cases of AML (75%), 8/24 cases of ALL (33%) and 66/73 cases of MDS (90%), with median percentages of positive blasts respectively of 15 (range 0-38), 0 (range 0-11) and 8 (range 0-69). In ALL the percentages of positive blasts were significantly lower than in AML and in MDS (p=0.0001). Survivin expression was unrelated in AML to the morphological subtype, in ALL to the immunological phenotype. In MDS with more than 5% bone marrow blasts percentages of survivin positive cells higher than in RA and RARS were observed (p=0.04). In MDS a significant inverse correlation between survivin and TUNEL positivity was identified by the Spearman correlation test (p=0.01), whereas survivin expression was independent of the proliferative rate. No significant relationship was observed between survivin levels and clinical and laboratory features such as age, leukocyte count or karyotype nor did survivin levels predict disease progression in MDS; among AML patients treated with intensive polichemotherapy, survivin expression was significantly higher in resistant cases (p=0.01). In conclusion, our findings confirm the high incidence of survivin expression in AML blasts and suggest that AML patients with high survivin levels are more likely not to respond to current therapy. Survivin abnor- mal expression also in MDS may play a role in promoting aberrantly increased cell viability and contribute to the altered homeostastic balance between cell growth and cell death, that seems to be one of the most important physiopathological mechanisms of MDS.

PO059
ERYTHROPOIETIN VERSUS ERYTHROPOIETIN + G-CSF FOR LOW-RISK MYELODYSPLASTIC SYNDROMES: PRELIMINARY RESULTS OF A RANDOMIZED CLINICAL TRIAL
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Recent clinical guidelines indicate a trial with hematopoietic growth factor(s) as appropriate in low-risk myelodysplastic syndromes (MDS) with low serum levels of endogenous erythropoietin; recombinant human erythropoietin (rEPO) at standard weekly doses of 30,000 IU has been largely used is this clinical setting since the early ‘90s, although more recently a combination therapy of rEPO and G-CSF has been claimed to allow for a higher response rate. Whether combined rEPO + G-CSF treatment is really superior to standard doses of rEPO alone is still debated. In particular, randomised studies comparing a combined approach with rEPO + G-CSF versus front-line standard doses of rEPO alone are still lacking. Present investigation was designed to compare, in a prospective, randomised study, the effects of standard doses of rEPO alone to the combination of rEPO plus G-
CSF in patients with low-risk (IPSS < 1) MDS. According to study design, low-risk MDS subjects with no previous treatment with growth factor(s) were randomly divided to receive rHEPO (10,000 IU 3 times a week sc.) or the same doses of rHEPO plus G-CSF (5 µg/kg b.w. twice a week sc.) for a minimum of 8 weeks. Patients unresponsive to rHEPO alone were offered the combination therapy for others 8 weeks, whereas subjects unresponsive to rHEPO + G-CSF were considered off study. Responders continued the treatment indefinitely. Between April 2001 and August 2002 26 consecutive subjects affected by MDS (16 males; 10 females; median age 75 years; 16 RA, 5 RARS, 5 RAEB with less than 10% bone marrow blasts) were enrolled into the study. Clinical characteristics of the two groups of patients at the start of the study were comparable. At May 31 2003, 24 patients were evaluable for response (12 in the rHEPO arm; 12 in the rHEPO + G-CSF arm). After the first 8 weeks of treatment, an erythroid response (major or minor) was observed in 5/12 (41.6%) patients treated with rHEPO alone, compared to 9/12 (75%) in the rHEPO + G-CSF arm. In 2/6 (33%) of the patients in the rHEPO arm unresponsive to rHEPO alone, addition of G-CSF induced an erythroid response at 16 weeks. Erythroid response has been maintained for at least 6 months in 4/12 (33%) patients treated with rHEPO alone and in 7/12 (58%) patients treated with the combination of rHEPO + G-CSF; all responders at 6 months were still responding and continuing the treatment with a median follow-up of 16 months. Progression of MDS into acute leukemia has been cumulatively observed in 4/24 (16%) patients (2 patients in each arm of the study). No adverse effects to therapy with growth factors were recorded in all the patients enrolled into the study. These preliminary data, although obtained from a relatively small cohort of MDS patients, seem to confirm the superiority of combination therapy with rHEPO plus G-CSF versus rHEPO alone in inducing an erythroid response in subjects with low-risk MDS, which is maintained long-term in the majority of them; the combination of rHEPO + G-CSF seems able to induce an erythroid response in at least a part of MDS patients unresponsive to rHEPO alone, without increasing the risk of leukaemic transformation.

PO060
INCREASED RISK OF THROMBOTIC EVENTS IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA RECEIVING ATRA TREATMENT
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All-trans retinoic acid (ATRA) has markedly improved the outcome of APL; however, the incidence of thrombotic events seems to be higher in the ATRA treated patients. To assess the true incidence of such complications, we compared 2 different cohorts of APL patients: 37 patients (M/F 17/20, median age 37 years, range 14 - 58) treated with chemotherapy (CHT) alone (GIMEMA 0389-group A) and 90 patients (M/F 40/50, median age 43.5 years, range 19-75) treated with ATRA + CHT (AIDA protocol-group B). In the group A, no patient had thrombotic complications during induction or consolidation treatment: however, among 15 patients who relapsed and received a reinduction treatment with ATRA±CHT, 3 (20%) developed a thrombotic disease [1 acute myocardial infarction (AMI), 1 pulmonary embolism, 1 deep venous thrombosis (DVT)]. In the group B, 11/90 patients (M/F 5/6, median age 60 years, range 32-71) (12%) had a thrombotic event. Of them, 8 patients developed it during induction (4 episodes of unstable angina, 2 right ventricular thrombosis, 2 DVT), after a median time of 26 days from ATRA treatment (range 3 - 78), a median PLTS count of 208×10⁹/L (3/8 patients showed a PLTS count < 50×10⁹/L when the thrombotic event occurred) and without prothrombotic biochemical abnormalities. The remaining 3 patients developed a DVT during consolidation phase, after 31, 34 and 37 days respectively from start of 1st consolidation cycle and with a normal PLTS count. Our data outline the increase of thrombotic risk linked to ATRA treatment in APL patients, with an incidence of 10-15% to be confirmed in other series: further prospective studies are warranted to analyse clinical and biological factors predisposing to thrombotic disease.
Many patients with inv(16)-positive acute myeloid leukemia (AML) achieve complete remission (CR). Using quantitative RT-PCR (qRT-PCR), we previously proposed critical CBFβ-MYH11 transcript copy number thresholds for prediction of relapse or cure. We now update the molecular follow up of our patients with inv(16) positive AML who were treated with ablative therapy and for whom cytogenetic and long-term (>36 months) molecular follow-up data are available. To facilitate comparison with data from other series, all our qRT-PCR data are now reported as CBFβ-MYH11/(ABL×10^4). Fifteen patients are currently alive either in first (n=12) or second (n=3) CRs lasting >36 months, supporting the concept that AML with inv(16) appears to be curable. Division of our qRT-PCR data into relapsing and non-relapsing subgroups revealed that all CBFβ-MYH11/(ABL×10^4) ratios <12 belonged to patients who maintained continuous long-term CR, whereas all ratios ≥25 belonged to patients who went on to relapse (difference between groups, p<0.0001 at Kruskal-Wallis test). Our extended follow up of the 5 patients with assays falling in the intermediate gray zone (ratios of 12–25) shows that the ratios of the 3 patients who maintained long-term CR fell and remained below the lower threshold (12 copies) associated with long-term CR, whereas the ratios of the other 2 patients returned above the upper threshold (25 copies) before relapse. Our extended molecular follow up further supports the validity of applying CBFβ-MYH11 transcript MRD thresholds evaluated by real-time RT-PCR to predict long-term CR or relapse in patients with inv(16) AML.

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We have previously observed that the level of minimal residual disease (MRD), as determined by flow cytometry, is an important prognosticator in adult patients with AML. In fact, patients with a number of residual leukemic cells after consolidation therapy >3.5×10^4 had a significantly higher relapse rate. In the present study we investigated to what extent autologous stem cell transplantation (ASCT) might impact on the level of MRD in patients with AML. Thirty-one adult patients with AML were entered onto the EORTC/GIMEMA AML11 protocol. Eleven of 31 (35%) patients were MRD+ at the end of consolidation therapy (median no. of residual leukemic cells 3.7×10^3, range 4×10^2–2.3×10^4); the remaining 20 (65%) were MRD- (median no. of residual leukemic cells 4.3×10^5, range 0–3.4×10^4). Since the median time from the completion of consolidation to ASCT was 53 days, MRD was investigated again within 7 days before transplantation, and, at this stage, only one case shifted from a negative to a positive MRD status. Therefore we evaluated 12 MRD+ (median no. of residual leukemic cells 5.9×10^3, range 6×10^2–2.7×10^4) and 19 MRD- patients (median no. of residual leukemic cells = 5×10^5, range 0–3.1×10^4). The difference between the two groups was statistically significant (p=0.000004). Within a median time of 368 days from ASCT all the 12 MRD+ patients and 5 (26%) of those MRD-relapsed. In 3 of these 5, relapse was preceded by increasing levels of MRD as observed on serial controls after ASCT. In the remaining 2 patients occurrence of relapse was unexpected since the serial controls never documented raising levels of MRD. The difference in relapse rate (100 vs 26%) between the MRD+ and MRD- group was highly significant (p=0.00004); the pre-ASCT MRD status was the factor most strongly associated with relapse risk in the multivariate analysis (p=0.0014). Importantly, there was no difference in the number of mononuclear cells infused per kilograms between the MRD+ and MRD- patients (median 3.84×10^10/kilograms, range 1.21–24.8 vs 5.27×10^10/kilograms, range 1.03–21.4, respectively), as well as between patients who relapsed and those who did not (median 3.5×10^10/kilograms, range 1.05–24.8 vs 5.73×10^9/kilograms, range 1.03–21.4, respectively). We conclude that 1) pre-ASCT MRD status predicts successive outcome in patients receiving ASCT; 2) high dose CHT conditioning regimen
followed by ASCT has no impact on the unfavorable prognostic value of high pre-ASCT MRD level; 3) sequential MRD monitoring post-ASCT may allow to predict impending relapse.

PO063
ANTICANCER ACTIVITIES OF HETRAZEPINES WEB-2086 AND WEB-2170 AS APOPTOTIC AND/OR CYTODIFFERENTIATING AGENTS OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS


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Background: PAF-receptor antagonists of the hetrazepine family, WEB-2086 and WEB-2170, have been previously shown to induce differentiation of both murine and human erythroleukemia cells and HL60 cells. Present study describes the apoptotic and/or cytodifferentiating effects of the two WEB-derivatives (WEBs) in acute promyelocytic leukemia (APL) cell lines and freshly-isolated leukemic blasts. Methods: NB4 and all-trans-retinoic acid (ATRA)-resistant (NB4-007-6 and NB4-M4R) APL cells as well as blasts from acute myeloid leukemia (AML) patients were incubated for different times with various concentrations of WEBs given alone or in combination with ATRA. Changes in cell mass and levels of expression of differentiation markers were evaluated by means of morphologic, biochemical and biochemical procedures including the NBT dye reduction test to monitor functional myeloid maturation. Induction of apoptosis was revealed by the DNA ladder assay and flow cytometry for annexin V. Activation of caspase 9, 8 and 3 in WEB-treated cultures was also evaluated by western blot.

Results: NB4 cells incubated with 0.5-1 mM WEBs underwent massive growth arrest and apoptosis with no appreciable differentiation. IC50 values after a 3-day treatment were 0.4 and 0.25 mM for WEB-2086 and WEB-2170, respectively. Comparable apoptotic effects of WEBs were observed in ATRA-resistant APL cells, NB4-007-6 and NB4-M4R, and also blasts of six patients with M1, M2 and M4 AML. Moreover, subapoptotic WEB concentrations acted synergistically with suboptimal amounts of ATRA (0.025-0.05 μM) and induced NB4 differentiation as shown by >85% and >60% increases in NB- and CD11b-positive cells, respectively. Conclusions: WEBs have been proven very effective as antiproliferative/apoptotic agents on ATRA-sensitive and -resistant APL-cell lines, and primary AML blasts. Such novel anticancer activities of WEBs are accompanied by their capability to enhance ATRA-induced differentiation potential and, eventually, high tolerability in vivo as demonstrated by the relative safe use of these agents to contrast PAF-mediated signals in animal models and human volunteers. On the whole, these findings have suggested that WEBs, given alone or in combination with other inducers, might represent a novel class of anticancer agents to improve the clinical treatment of APL and, possibly, other types of leukemia.

References

PO064
CD34+ ACUTE MYELOID LEUKEMIA IS A HETEROGENEOUS SUBSET WITH DIFFERENT LEVELS OF APOPTOSIS AND PROGNOSIS


Several experimental and clinical studies have confirmed the control of chemotherapy induced apoptosis by bcl-2 and bax oncoproteins in acute myeloid leukemia (AML). Therefore, bcl-2 measured in conjuction with bax (bax/bcl-2 ratio) could provide valuable information on intrinsic chemosensitivity in AML. Their quantitative determination might be a very useful tool to predict the clinical response and outcome to apoptosis-inducing drugs. Furthermore, it has been demonstrated a striking correlation between a low apoptosis and immaturity, suggesting that the poor prognosis of AML patients is essentially due to the lack of hematopoietic differentiation. On this background, literature data have generally considered CD34 antigen as a sign of an unfavorable outcome. Therefore, the aims of our study were: 1) to quantify bax/bcl-2 ratio in CD34+ AM L patients by flow cytometry; and 2) to evaluate its independent prognostic value within this AML subset, demonstrating that immaturity and apoptosis block are not a synonymous. We investigated bcl-2 and bax expression in 158 CD34+ AML de novo patients, except FAB M 3, median age 63 years, treated with intensive
is important for regulation of cell proliferation, partic-
sor gene that encodes a 1863 amino acid protein that
of tumor suppressor genes. BRCA1 is a tumor suppres-
much as DNA repair/recombination processes related
to the maintenance of genomic integrity, induction of
apoptosis in damaged cells and regulation of tran-
scription. Recent data reveal that promoter hyperme-
thylation is frequently associated with BRCA1 inacti-
non-inherited breast and ovarian carcinomas. Using a
methylation-specific PCR, we studied BRCA1
promoter hypermethylation in 80 patients (40 females,
40 males, median age 63.5 years, range 16-85 years),
with acute myeloid leukemia (AML). BRCA1 was hyper-
methylated in 42/80 (52.5%) AML samples. No differ-
ences were noted when looking at patient’s character-
istics, including age, gender, FAB-subtype and therapy-
related versus de novo AML. Karyotype was available for
53 patients. The frequency of BRCA1-methylation was
higher in patients with cytogenetic abnormalities, when
compared to those with normal cytogenetics (19/29,
65% versus 9/24, 37%, respectively, p=0.05). In partic-
lar, when grouping patients according to cytogenetic
risk groups, as defined by Grimwade et al. (Blood, 1998),
we found a significantly higher incidence of aberrant
BRCA1 methylation in patients with a karyotype asso-
ciated with adverse prognosis, compared to patients
with intermediate or favourable karyotype (6/6, 100%,
14/34, 41% and 8/13, 61%, respectively, p=0.02). This
study points to a possible link between genetic insta-
ability related to BRCA1 inactivation and cytogenetic
abnormalities in adult AML.

PO067 DARBEPOETIN IMPROVES ANEMIA AND QUALITY OF LIFE IN MYELODYSPLASTIC SYNDROME: A PILOT STUDY
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There is much concern today about the formation of anti-erythropoetin antibodies in patients treated with
subcutaneous recombinant human erythropoetin
(rHuEPO) reported in patients with chronic renal fail-
ure (N Engl J Med, 2002). Since the prevalence of ure-
emia increases with age, it occurs frequently as a con-
comitant disease in elderly patients with myelodys-
plastic syndrome (MDS). Darbepoetin α is a hypergly-
cosylated rHuEPO analogue. We evaluated its effects on
anemia in a pilot subset of patients. Eight consecutive
patients with refractory anemia and chronic renal fail-
ure were included in the study to receive an initial dose of
darbepoetin 150 µg s.c. weekly to be increased to
300 µg in non-responders. Response criteria were
defined as follows: complete response if Hb rise of ≥2
g/dL or if no transfusion requirement in transfusion-dependent patients, partial response if Hb rise ≥ 1g/dL to < 2 g/dL or 50% reduction in transfusion requirement. Quality of life changes were measured using the QOL-E questionnaire (Oliva et al., 2002). Median age of patients was 78 (range 59-91) years. Baseline serum erythropoietin levels were median 72 (range 21-225) IU/mL. Six patients were transfusion-dependent, requiring 1 to 3 monthly transfusions. Two patients did not require transfusions and had baseline Hb values of 8.9 and 10.2 g/dL, respectively. Seven patients had a follow-up of at least two months. Of the 5 transfusion-dependent patients, 1 was excluded from the response analysis because of metastatic bone disease discovered after the study entry. Three of the remaining 4 patients obtained a 50% reduction of transfusion requirement at 150 mcg and a complete response after dosage increase. Two patients became transfusion-dependent again after 5 months from the start of study. Of the 2 patients that were not transfusion-dependent, both obtained a complete response after 2 months of therapy at a dosage of 150 µg and 300 µg, respectively. At univariate ANOVA analysis, the change in the hematology parameters were associated with significant improvements in quality of life treatment-outcome index (QOLE-TOI) scores (p=0.003). No side effects were observed during study period. In conclusion, darbepoetin is safe and well-tolerated in patients with myelodysplastic syndrome. Therapeutic response is associated with improvement in quality of life. This pilot study suggests that darbepoetin is effective for the treatment of anemia in myelodysplastic syndrome. Therapeutic response is associated with improvement in quality of life. This pilot study suggests that darbepoetin is effective for the treatment of anemia in myelodysplastic syndrome. Therapeutic response is associated with improvement in quality of life. This pilot study suggests that darbepoetin is effective for the treatment of anemia in myelodysplastic syndrome. Therapeutic response is associated with improvement in quality of life.
Chronic myelomonocytic leukemia (CMML) is distinguished by wide heterogeneity of clinical and hematologic characteristics at presentation, sharing features of both myelodysplastic syndromes (MDS) and myeloproliferative disorders (MPD). An arbitrarily chosen leukocyte count has been recently used to differentiate between a ‘dysplastic’ type (M-D-CMML, with \(<12\times10^9\) WBC/L) and a ‘proliferative’ type (M-P-CMML, with \(>12\times10^9\) WBC/L), while a most recent proposal by the WHO included CMML in a new category of MDS/MPD disorders. However, no general consensus has been reached on this classification dilemma. Very little is known on the biology of CMML, and no treatment has proven effective in modifying the natural course of the disease. By standard morphology, multiparameter flow cytometry, RAS genes sequencing, and clonogenic assays, we investigated PBMC of 11 consecutively newly diagnosed CMML patients admitted at Ospedale Maggiore of Milan from 11/2002 to 04/2003. Our series included 5 males and 6 females, with a median age of 71 years. Median WBC value was 7.5\times10^9/L (range 3.7 to 17.2), while differential showed median monocyte values of 2.7\times10^9/L (range 1.0 to 4.98) and 27% (range 14% to 55%). Eight out of the eleven patients had WBC \(<12\times10^9/L\), being classified as M-D-CMML. All patients had normal karyotype and were negative for the presence of point mutation on codons 12, 13 and 61 of N- and K-RAS. After density gradient-based mononuclear cell enrichment, CD14+ cells were sorted by immunomagnetic techniques (MiniMACS cell separator). Cell immunophenotype was determined by the following combinations of antibodies: CD14/CD19, CD14/CD34, CD4/CD8, CD14/CD3, CD16/CD56, CD15/CD14, and CD33/CD14. Six healthy donor blood specimens were simultaneously analyzed as a control. Cell cultures were performed on unseparated PBMC, on the CD14+-enriched and on CD14+-depleted fractions, with or without the addition of growth factors (GFs) [SCF, GM-CSF, IL-3, Epo]. While spontaneous growth was observed both in normal controls and CM ML, the CFU-GM number was higher in the MP-CM ML subgroup, with or without the addition of GFs. A similar pattern of growth was observed in the CD14+-depleted specimens, whereas no colony growth was ever observed in CD14+-selected fractions, either in normal controls nor in CMML. The addition of GFs did not significantly affect colony number in CMML, whereas it did in normal controls, especially in CD14-depleted fractions. The number of BFU-E was considerably higher in normal controls than in CMML. FACs analysis showed a significantly higher expression of CD14, CD15, CD33, and CD16/CD56 in CMML than in normal controls. No difference was observed between MD- and MP-CMML. Analysis of correlations unveiled positive association between CD34, and all lymphocytic markers. Among hematologic characteristics, hemoglobin levels correlated inversely with CD14, CD16/CD56, and LDH serum levels. BFU-E correlated positively with CD34, CD4, CD8, CD19, and CD3 expression. Although this study was performed on a limited number of samples, in CMML immunophenotypic and in vitro growth pattern characterization of circulating cells seems to correlate with important clinical characteristics and could be useful for further investigation on physiopathology of the disease and development of new therapeutic agents.
chemotherapy, and rapid disease progression. The 3q26 locus, which is rearranged in up to 5% of myeloid malignancies (AML, MDS, MLC), participates in t(3;3) and encodes for two proteins involved in chromosomal abnormalities in leukemias, the EVI1 gene and MDS1, which are expressed separately or as a combined transcript, throughout an alternative splicing. EVI1 encodes a zinc finger DNA-binding protein and its transformation can block terminal differentiation of granulocytes and erythrocytes. It is expressed at extremely low levels in normal peripheral blood and bone marrow, while in AML with inv(3) or t(3;3), its transcription is elevated. Instead TPO gene, mapped on 3q26.33-q27, has not been found to be activated in leukemia cells from patients with the 3q21.3q26 syndrome. Moreover, it has been shown that there is a significant association between EVI1 expression and unfavorable or complex karyotypes and that overexpression of EVI1 correlates with poor treatment outcome. The hypothesis of a chromosome 3 instability, maybe associated with EVI1 expression and rearrangements of genes interfering with megalakaryocytopenia, could be raised. Recently, two patients affected by RAEB and RAEB-t, with inv(3), obtained CR with a normalization of trilineage dysplasia after treatment with arsenic trioxide, thus suggesting a possible interaction of this drug with the EVI1 gene which is located in that region.

PO070
HUMAN RECOMBINANT ERYTHROPOIETIN + DIFFERENTIATING AGENTS IN THE TREATMENT OF LOW-INTERMEDIATE-RISK MYELODYSPLASTIC SYNDROMES
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Hyporigenerative anemia is quite common in myelodysplastic syndrome (MDS) patients and anemia-related complications represent a frequent cause of morbidity and death. Human recombinant erythropoietin (rEPO) can substantially improve anemia in about 25-45% of MDS patients with bone marrow (BM) blast <5% (low-intermediate risk); however, response rate is lower in transfusion-dependent patients and in those with BM blast excess (RAEB). In our previous study (Ferrero et al; Leuk Res 1996; 20, 867-876) a combination of differentiating agents: 13-cis retinoic acid (RA) + 1,25alphah(OH)2vitamin D3 (D3) significantly reduced transfusion requirement in 30-40% of anemic MDS patients. We wished now to test the combination of differentiating agents + rEPO in the hope to improve the response rate. Twenty-one low-intermediate risk MDS patients (10 RA, 1 RARS, 2 "refractory cytopenia with multilineage dysplasia", 2 RA with "5q-syndrome", 6 RAEB 1 according to WHO classification) entered the study. Median age was 73 (46 - 91); prognostic score (IPSS), evaluable in 16, was: "low risk" in 3, "intermediate 1 risk" in 13, intermediate 2 risk in 1 patient, respectively. All but one patient had Hb values <9 g/dL and 18/21 were transfusion-dependent (median 2 U/month, range 1-7). Endogenous serum EPO levels were evaluated in 11 patients and found > 200 U/l in 3. All patients received a combination of RA + D3 (Roaccutol 20-40 mg/day and Rocaltrol 1 microgram/day, kindly provided by ROCHE) + rEPO (30,000 - 80,000 U/week in 2-5 doses); 3/6 RAEB patients also received intermittent, low dose 6-thioguanine. Erythropoietin response was defined as: major in the case of no transfusion requirement for at least 3 months or Hb increment >2 g/dL, minor in the case of >50% reduction in transfusion requirement or 1-2 g/dL Hb increment. An erythroid responses was observed in 12/21 patients (57%). In particular, 9/15 patients (60%) with BM blast <5% responded (6 with a major, 3 with a "minor" response), while a minor response was observed in 3/6 RAEB 1 patients. A response was evident in 9/18 (50%) transfusion-dependent and in all 3 untransfused patients, in 7/8 patients with serum EPO level <200 U/l and in 0/3 with higher levels. Median response duration was 10 months (1+-20+). At a median follow up of 21.5 months, 12/15 patients without blast excess and 2/6 with RAEB are alive. In conclusion, the combination of rEPO + differentiating agents may be more efficient than each treatment alone in improving anemia, particularly in patients without blast excess.

PO071
TRANSFUSION NEED INDICATORS IN DYSPLEASTIC HEMOPOIESIS: RETROSPECTIVE ANALYSIS ON 50 PATIENTS
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In myelodisplastic syndromes (MDS) anemia level is imputable to blast bone marrow infiltration and ineffective hemopoiesis. Hemoglobin concentration is frequently used in MDS prognostic assessment systems. Transfusion need, depending from patient’s clinical conditions and hemoglobin level, is indirectly but quite accurately related to myelodysplastic pathology prognosis. We try to identify transfusion need indicators in dysplastic hemopoiesis. With this purpose we used various laboratory parameters well-known as MDS prognostic factors or new parameters not formerly consid-
We performed IRF (r-0.74) and MRF (r-0.68), immature reticulocyte fraction (IRF) were performed by ADVIA 120-> (BAYER, Diagnostic Division, Tarrytown, NY) and ABBOTT CELL DYN 4000-> (Abbott Diagnostics, Santa Clara, Ca, USA), and reticulocyte fractions were performed by ADVIA 120-> (BAYER, Diagnostic Division, Tarrytown, NY, USA) and ABBOTT CELL DYN 4000-> (Abbott Diagnostics, Santa Clara, Ca, USA). Correlation between tested parameters and transfusion need was performed by Pearson’s r test and R² test. We found that IRF (r-0.74), intermediate fluorescence reticulocyte fraction (MFR, r=0.81) and high percentage of bone marrow eosinophils (r=0.59) correlate with a higher effective hemopoiesis and with a lower erythrocyte transfusion need. High value of circulating erythroblasts correlate with high platelets (r=0.56) and red blood cells (r=0.68) transfusion need, but high medullary erythroblasts percentage correlate only with an high red cell transfusion need (r=0.68). It’s interesting to remark how, in our study, high levels of circulating IRF and MRF correlate with high medullary eosinophils percentage and with low number of bone marrow erythroblasts. This may suggest that IRF and MRF correlate with a greater effective erythropoiesis. All mentioned parameters are normally present in common MDS follow-up tests and easy and not expensive to perform. Above mentioned indicators may be employed to: 1) correlate identified indicators with patients survival; 2) identify patients with greater transfusion need; 3) stratify patients in homogeneous groups which can be submitted to different therapy regimens (supportive, differentiative, cytotoxic); 4) monitor response to therapy. Evolution of this study is to verify validity of these indicators on a larger population of dysplastic patients.

Overall, about 20% of patients with myelodysplastic syndromes (MDS) improve their hemoglobin levels using recombinant erythropoietin (r-EPO). Usually, subjects with no or low need of transfusions and reduced levels of endogenous EPO have the best probability of responding. Thalidomide may induce erythroid responses in about 30% of MDS. Interestingly, these responses may be achieved even in transfusion-dependent patients with high serum EPO levels, a subset of MDS patients with very low chance to respond to r-EPO, alone or in combination with G-CSF. This suggests that the mechanisms of action of the two drugs are probably different and that, therefore, a synergistic effect could be possible. Thus, we designed a pilot clinical trial to explore the potential therapeutic effects of the combination of r-EPO plus thalidomide in transfusion-dependent MDS patients, previously unresponsive to these drugs employed as single agents (step 1). All patients are planned to receive r-EPO (Epoietin-α, Eprex, Ortho-Biotech, or Globuren, Dompe'-Biotec; Epoietin-β, Neo-Recombin, Roche) at the dose of 40.000/U s.c., once-weekly, in combination with thalidomide (Thalidomid, Grunenthal) 100 mg/d p.o. for one week, to test tolerance, and then 200 mg/d (step 2). Patients who do not evidence an erythroid response (IWG criteria), stop the treatment after 12 weeks. In order to verify the real synergy of the association or the simple efficacity of the new drug adjuncted, after 12 weeks of combined therapy responders continue with a) only thalidomide (if previously treated with r-EPO alone) or, alternatively, with b) only r-EPO (if previously treated with thalidomide alone)(step 3). Patients who then lack their response, re-start again combined therapy (step 4). Sixteen patients with low-to-intermediate-1 IPSS risk (10 males, 6 females; median age 62 yrs, range 24-78; 10 RA, 5 RARS, 1 RAEB-1, according to WHO criteria), have been so far selected within two cohorts of previously described patients unresponsive to r-EPO (n. 8) or thalidomide (n. 8) alone (step 1). To date, 8 patients (4 for each group) have completed step 2 (12 weeks of combined therapy) and are evaluable for response, while 2 additional patients have interrupted early the trial because of relevant neurological side effects likely due to thalidomide. Reduction of thalidomide dose to 100 mg/d was necessary in further 2 patients. Two patients within the group of those unre-
Angiogenesis is regulated by a balance of various positive and negative angiogenic molecules. During tumorogenesis, the vasculature becomes activated to grow new capillaries in response to appropriate stimuli. Angiogenesis has been proposed as a useful prognostic factor in melanoma, breast, prostate, gastrointestinal, and ovarian cancers. A pathogenic role has been proposed for angiogenesis in a number of hematologic malignancies, in which anti-angiogenetic approaches are currently being investigated. In particular, a significant increase of microvessel density (MVD) and endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), tumor necrosis factor (TNF) and hepatocyte growth factor (HGF) have been described in CML, AML, ALL and MDS. In particular, bFGF is a mitogen for hematopoietic and fibroblastic growth factor levels and circulating endothelial cells.

**PO073**

ANGIOGENIC CYTOKINES IN THE PERIPHERAL BLOOD OF MYELODYSPLASTIC SYNDROME PATIENTS: CORRELATION OF BASIC FIBROBLASTIC GROWTH FACTOR LEVELS AND CIRCULATING ENDOTHELIAL CELLS

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Angiogenesis is regulated by a balance of various positive and negative angiogenic molecules. During tumorogenesis, the vasculature becomes activated to grow new capillaries in response to appropriate stimuli. Angiogenesis has been proposed as a useful prognostic factor in melanoma, breast, prostate, gastrointestinal, and ovarian cancers. A pathogenic role has been proposed for angiogenesis in a number of hematologic malignancies, in which anti-angiogenetic approaches are currently being investigated. In particular, a significant increase of microvessel density (MVD) and soluble angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), tumor necrosis factor (TNF) and hepatocyte growth factor (HGF) have been described in CML, AML, ALL and MDS. In particular, bFGF is a mitogen for endothelial cells and fibroblasts in vitro and a potent inducer of angiogenesis in vivo. Therefore, we investigated the role of bFGF and correlated its levels with circulating endothelial cells and endothelial precursors, in a cohort of 25 MDS patients and 10 normal controls. Clinical characteristics of MDS patients are distributed as follows: M/F 13/12, mean age 75 (range 27-101), FAB subtypes: RA 9, RARS 5, RAEB 5, RAEB-t 4, AM L 1, CM ML 1, IPSS classes: Low 9, INT-1 8, INT-2 4, HIGH 4. bFGF levels were measured by ELISA immunoassay (R&D Systems, Minneapolis, USA) on patients sera. Resting CECs were defined by means of immunocytofluorimetric assay, as negative for CD45, CD105, CD106, CD133 and positive for P1H12, CD31 and CD34. Activated CECs were defined as CD45+, CD133+ and CD34+, CD31+, P1H12+, CD105+ or CD106+. CEPs were positive for CD133 (Mancuso et al, Blood 2001). Mean values of bFGF are 9.57 pg/mL (SD±7.77) for MDS patients, and 1.51 pg/mL (SD±1.25) for healthy controls. No correlations with survival was evidenced. Nevertheless, the levels of bFGF resulted to directly correlate with total and activated CECs (p<0.003; p<0.013, respectively, Spearman correlation). Our results suggest that bFGF may have a specific role in MDS angiogenesis. This finding is particularly intriguing as new therapeutic approaches that specifically target bFGF are currently available, such as monoclonal antibody and antisense cDNA.

**PO074**

HIGHER LEVELS OF SOLUBLE VASCULAR ENDOTHELIAL GROWTH FACTOR-2 IN MYELODYSPLASTIC SYNDROME PATIENTS ARE ASSOCIATED WITH LOW AND INTERMEDIATE-1 IPSS RISK CLASSES

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Recently, angiogenesis has been proposed as a useful prognostic factor in solid cancers and in hematologic malignancies, in which anti-angiogenetic approaches are currently being investigated. In particular, a significant increase of microvessel density (MVD) and soluble angiogenic factors, including endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), tumor necrosis factor (TNF) and hepatocyte growth factor (HGF) have been described in CML, AML, ALL and MDS. In particular, a prognostic role of VEGF intracellular levels has been proposed in AM L and in chronic lymphocytic leukemia (CLL). The VEGF receptor family in mammals contains three members, VEGFR1, VEGFR2 and VEGFR3, which directly regulate the formation of blood and lymphatic vessels. The expression/function of VEGFR1 and VEGFR2 in hematopoiesis is under scrutiny. Temporal and regional activation of VEGF/VEGFR2 signaling pathways seems to be critical for mobilization and recruitment of HSCs and CEPs and may play a role in the physiology of postnatal angiogenesis and hematopoiesis. Therefore, we investigated the role of VEGF and of its soluble receptor VEGFR2 (sVEGFR2), in a cohort of 25 MDS patients and 10 normal controls. Clinical characteristics of MDS patients were distributed as follows: M/F 13/12, mean age 73 (range 27-101), FAB subtypes: RA 9, RARS 5, RAEB 5, RAEB-t 4, AM L 1, CMML 1, IPSS classes: Low 9, INT-1 8, INT-2 4, HIGH 4. Cytokines were
measured by ELISA immunoassay (R&D Systems, Minneapolis, USA) on patients sera. Mean values of VEGF for MDS patients and healthy controls are respectively 183.07 pg/mL (SD±201.45) and 206.33 pg/mL (SD±186.05). Mean values of sVEGFR2 are 7684.66 pg/mL (SD±1804.44) for MDS patients, and 8078.91 pg/mL (SD±930.49) for healthy controls. We have not found significative differences between MDS and healthy controls as far as VEGF and sVEGFR2 levels are concerned. When comparing the cytokines levels among the different FAB and IPSS subclasses, sVEGFR2 resulted significantly higher in the low/INT-1 IPSS risk classes ($p=0.041$, Mann-Whitney test). After a median follow-up of 361 days (range 37-412 days) from the analysis, 19/25 patients were alive. When we evaluated the number of CECs, CEPs and the levels of VEGF and sVEGFR2 in the group of living patients and in the group that had died (19 vs 6), the levels of sVEGFR2 were significantly higher in the patients that have survived ($p<0.02$, Mann Whitney test). sVEGFR2 level was is higher in the lower risk patients and in the survivor group, and its possible prognostic role should be further investigated in this pathological setting.

MYELOMA AND PLASMA CELL DYSCRASIAS

P0075
TOLERANCE TO LONG-TERM THALIDOMIDE THERAPY FOR PATIENTS WITH ADVANCED AND REFRACTORY MULTIPLE MYELOMA
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Thalidomide is remarkably active in advanced relapsed and refractory multiple myeloma (MM), so that its use has been recently proposed either in newly diagnosed patients or as maintenance treatment after conventional or high-dose therapy. This latter therapeutic approach has risen the concern of side effects of long-term therapy with this drug. In order to investigate this issue we analyzed the outcome of 32 patients (26M, 6F, median age = 61.5 yrs) who received salvage therapy with thalidomide ± dexamethasone for longer than 12 months (median 15 months, range 12 to 35 months) at our Center. All the patients had achieved at least a minor response (> 25% decrease in serum or urine M component) upon treatment with thalidomide alone (200mg/day, n= 14) or thalidomide (200mg/day) and dexamethasone (40mg/day for 4 days every 4 weeks) (n=18). Thalidomide was initially well tolerated in all these patients; grade II constipation was present in a minority (15%) of the patients. Upon long-term treatment, however, neurotoxicity was the most troublesome and frequent toxic effect that was observed, the incidence averaging 69%. Among these 22 patients symptoms included paresthesias, tremor and dizziness. Neurotoxicity was grade 1 in 8 patients (36%); grade 2 in 9 patients (41%), thus determining thalidomide dose reduction to 100mg/day; and grade 3 in 5 patients (13%) who had subsequently to interrupt therapy despite their response. In these latter patients electromyographic study revealed mixed sensorimotor peripheral neuropathy. The incidence of neurotoxicity was comparable in patients receiving either thalidomide alone or thalidomide + dexamethasone. These results suggest that long-term thalidomide therapy in MM may be hampered by the remarkable neurotoxicity of the drug, that undergoes only minimal regression after treatment withdrawal. Studies addressing the issue of the role of thalidomide as maintenance therapy for MM are currently underway. In the interim, alternative dosages and schedules of treatment with thalidomide should be investigated.
PO076
DIFFERENTIAL CLINICAL CHARACTERISTICS BETWEEN IGM MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOULDERING WALDENSTROM’S MACROGLOBULINEMIA

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Introduction: As recently proposed by the Waldenström’s macroglobulinemia (WM) Study Group, in the absence of symptoms and irrespective of the size of IgM monoclonal gammopathy (MG) the unequivocal evidence of bone marrow (BM) lymphoplasmacytic (LPC) lymphoma is the only characteristic able to distinguish smouldering WM (SWM) from IgM MG of undetermined significance (MGUS). Whether on the basis of this classification clinical distinctive features exist between the two pathological entities has not been still investigated. Patients and Methods: IgM MGUS was diagnosed in the presence of any IgM size, less than 10% BM LPC infiltration, and no evidence of overt lymphoproliferative disease for at least 12 months from diagnosis; SWM diagnosis required any IgM size, more than 20% BM LPC infiltration with intertrabecular (nodular, interstitial or diffuse) pattern, and no treatment requirement (i.e., development of constitutional symptoms, cytopenia(s), organomegaly, and/or symptoms related to the monoclonal component) for at least 12 months since initial observation. On the basis of these criteria, 138 IgM MGUS patients and 34 SWM patients were enrolled in the study. To compare baseline clinical characteristics according to diagnosis, Mann Whitney U test and Fisher exact test were used for continuous and categorical variables, respectively. Results: The median serum IgM monoclonal component and the erythrocyte sedimentation rate were significantly lower in the IgM MGUS group ([0.9 g/dL (range: 0.14-2.9) and 28 mm/h (range: 2-123), respectively] than in the SWM group [1.3 g/dL (range: 0.2-6.62) and 70 mm/h (range: 17-130)] (p<0.0003 and p<0.0001, respectively). On the contrary, the median hemoglobin level was significantly higher in the IgM MGUS group (14.2 g/dL, range: 8.1-17.9) than in the SWM group (13 g/dL, range: 10-16.7)(p<0.003). The proportion of IgM MGUS patients with one and two polyclonal serum immunoglobulin reductions, respectively, was significantly lower than that of SWM patients (7.5% vs 29.4% and 1.5% vs 8.8%, respectively)(p<0.002). The median serum β2-microglobulin level was higher in the SWM group (2.24 µg/mL, range: 1.3-9) than in the IgM MGUS group (2.0 micrograms/mL, range: 0.5-7.9) with a borderline p-value (=0.06). Age, sex, the% of patients with detectable Bence Jones proteinuria, absolute neutrophil counts, absolute lymphocyte counts, and platelet counts did not differ significantly between the two patient populations. At a median follow up of 80 months (range: 12-195), 14 IgM MGUS (10%) evolved to malignant lymphoproliferative disease (overt WM, n=13; IgM multiple myeloma, n=1), the median time from diagnosis being 75 months (range: 12-117). At a median follow up of 64 months (range: 12-204), 13 SWM (38%) evolved to symptomatic WM, the median time from diagnosis being 55 months (range: 13-154). Event free survival of IgM MGUS patients at 5 and 10 years was 95% (95%CI, 87-98%) and 83% (95%CI, 71-90%), respectively; event free survival at 5 and 10 years of SWM patients was 77% (95%CI, 56-89%) and 42% (95%CI, 19-64%), respectively. Conclusions: IgM MGUS and SWM are pathological entities with distinctive clinical characteristics that must be taken into account besides BM features. This seems relevant in view of the very different natural history and outcome of these two subgroups of asymptomatic IgM MG.

PO077
ALLOGENEIC TRANSPLANTATION AFTER NONMYELOABLATIVE CONDITIONING CONSOLIDATION THERAPY FOLLOWING HIGH-DOSE MELPHALAN AND AUTOLOGOUS STEM CELL TRANSPLANTATION IMPROVE OUTCOME IN HIGH-RISK MULTIPLE MYELOMA

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The benefit of allografts in high-risk MM pts is mainly mediated through a graft vs. myeloma (GvM) effect. However, allografts have been hindered by high treatment-related mortality (TRM). To reduce the treatment-related mortality but retain the cytoreductive effect, we designed a tandem transplantation program consisting of high-dose chemotherapy supported by autologous stem cell transplantation (auto-SCT) followed by a dose-reduced conditioning regimen with allogeneic stem cell transplantation (allo-SCT) to induce a GVM effect. We treated 26 pts, 12 males and 14 females, with a median age of 49 years (range 36-62). While 10 pts were enrolled after relapse from previously treatment (5/10 received previously single or double HDM with autoSCT), the tan-
The median number of allo-CD34+ cells infused was 4.5 ×10^6/kg (range 1.2-10.0) and CSP/MTX was given to control graft rejection and GVHD. No myelosuppression or mucositis were observed and no pts received transfusional support. Acute GVHD was diagnosed in 10 pts (5 grade I-II; 5 III-IV). Chronic GVHD was observed in 6 pts (1 extensive; 4 limited). Donor lymphocyte infusions were given to 9 pts either to attain full donor chimerism or to eradicate residual disease. Ten pts remained in continuous CR according to the EBM criteria with negative immunofixation; 8 pts had a PR, most of those with still decreasing monoclonal band. With a median follow-up of 19 mo, 20 pts still alive while 6 pts have died because of aGVHD (1 pt.) or progressive disease (5 pts). The actuarial survival was 69.7 ±11% and the progression-free survival was 63.7 ±11%. Interestingly, the progression-free survival probability was 69.3 ±17% in 16 pts who received more than 4.0 ×10^6/kg allogeneic CD34+ cells as compared to 48.0 ±16% in 10 pts who received a minor allogeneic CD34+ cell dose. These data show that NST induces excellent disease control in MM, after reducing tumor burden with HDM and autologous stem cell support. Also, this combined autografting-allografting is well tolerated in older pts with a low TRM. GVHD is the single most serious complication.

In patients with MM a number of recurrent translocations involving chromosome 14 at band q32 have been recently identified, the most common being the t(11;14) and the t(4;14). Both these chromosomal abnormalities are closely associated with particular presenting features of the disease and may help to identify patients at different risk of death; in particular, the t(11;14) predicts for good prognosis, whereas the t(4;14) has been recently reported to be associated with an unfavorable clinic outcome. The t(4;14) affects at least two potential oncocenes, MMSET on der(4) and Fibroblast Growth Factor Receptor 3 (FGFR3) on der (14); the role of both these genes in the pathogenesis of MM has not been fully elucidated. In the present study we investigated the frequency and the prognostic relevance of the t(4;14) in a series of 63 patients with de novo MM, who were randomized to receive either a single (Tx-1) or a double (Tx-2) autotransplant as primary therapy for their disease. For this purpose we analyzed (1) the presence of t(4;14) by RT-PCR of the hybrid transcript between MMSET/IgH and the IgH locus; (2) the overexpression of FGFR3 by Real-time RT-PCR; (3) the frequency of potentially activating point mutations in the FGFR3 translocated coding region, by direct sequencing of RT-PCR products; 4) the relationship between t(4;14), response to high-dose therapy and outcome of autotransplant(s). Overall, the t(4;14) was detected by RT-PCR in 17/63 patients (27%); of these patients, 13 had both MMSET/IgH fusion gene and FGFR3 overexpression, while 4 patients had MMSET/IgH but did not overexpress FGFR3. This finding further confirms the possible discrepancy between MMSET/IgH positivity and FGFR3 overexpression. Comparison between t(4;14) positive and t(4;14) negative patients revealed that both groups were well balanced with respect to the most common presenting features of MM. In 36 patients, for whom material was available, FISH analysis for the detection of 13q deletion and/or monosomy was performed. Results showed that t(4;14) positive patients were more likely to carry also del(13) than t(4;14) negative patients (46% vs. 29%, respectively). On an intention-to-treat basis, the probability of attaining stringently defined complete remission following either Tx-1 or Tx-2 was significantly lower for t(4;14) positive patients in comparison with t(4;14) negative patients (6% vs. 35%, respectively; p= 0.05). With a median follow-up of 40 months, no difference in overall survival (OS) was detected between the two groups. At the opposite, in comparison with the t(4;14) negative subgroup, patients carrying the t(4;14) had significantly lower event free survival (EFS) (23 vs. 32 months, respectively; p = 0.01) and duration of remission (23 vs. 32 months, respectively; p = 0.01). It is concluded that the t(4;14) was an unfavorable prognostic factor for previously untreated patients with MM undergoing single autologous transplantation or double autologous transplantation. The presence of point mutations in the FGFR3 coding region detectable in a subgroup of patients carrying the t(4;14) suggests a possible constitutive FGFR3 activation.

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PO079
INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION (FISH) DETECTION OF CHROMOSOME 13 ABNORMALITIES (DEL 13) IN MULTIPLE MYELOMA


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Chromosome 13 abnormalities have been associated with an unfavourable prognosis in MM patients. Studies using conventional cytogenetic chromosomes techniques rarely detect neoplastic metaphase cells in monoclonal gammopathies. To circumvent the low proliferative activity of myeloma cells, interphase FISH has been assessed. Between October 2002 and April 2003, bone marrow aspirates from 41 patients undergoing investigation for monoclonal gammopathy were analysed for del13 by FISH. Twenty-four patients were MM at diagnosis, 15 at relapse, 2 were MGUS. Plasma cells were positively selected from aspirates by means of anti-CD138-coated magnetic beads (Milenyi Biotech, UK) and their purity checked by citofluorimetric analysis of PE anti-CD38 stained cells. The percentage of plasma cells exceeded 85% for all patients. Nuclei from purified fixed plasma cells were prepared for interphase FISH. Chromosome 13 deletions were identified by means of a LSI13RB1 probes (Vysis, UK). For each sample 200 plasma cells were scored. Del13 was identified in 65% of MM and in both cases of MGUS. Among relapsed patients del13 was found in 86%; in MM at diagnosis its incidence was 50%. There was no significant difference in the prevalence of del13 according to age, % of BM plasma cells, B2M, clinical stage and immunoglobulin phenotype. One MM patient showed a biallelic loss of signals involving 13p11.2. The detection of del13 by FISH should be incorporate in all future trials for accurate patients stratification, according to prognostic factors.

PO080
COMMON AND RARE EFFECTS OF LOW-DOSE THALIDOMIDE IN MULTIPLE MYELOMA: TOWARDS THE OPTIMIZATION OF THE RISK/BENEFIT RATIO


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Thalidomide (Th) has demonstrated a remarkable activity in the treatment of multiple myeloma (MM), but unfortunately it often causes several toxicities. We investigated its common and rare side effects, especially analyzing peripheral neuropathy (PN), in order to seek the dose minimizing toxicity without affecting response rate. From May 2000 to July 2002 fifty-nine patients, with MM (50 relapsed, 5 stable, 4 newly diagnosed), received Th alone or combined with oral Melphalan (Melph), at a dose of 100 mg/day, escalated weekly by 100 mg increments until a maximum dose of 400 mg/day. Melph was given at a dose of 0,20 mg/kg/day for four days every 28 days. The median dose (MDD) of Th was defined as the ratio between cumulative dose given until PN onset and days from enrolment until PN appearance. Time to peripheral neuropathy (TTN) indicated time from the start of therapy to PN onset. Sixteen patients (27%) discontinued Th because of its toxicity. The commonest non-hematological side effects not dose and time dependent until 400 mg/day were constipation (71%), somnolence (36%) and fatigue (20%), while the most rare ones were deep venous thrombosis (7%), dizziness (7%), confusion (5%), bradycardia (5%) and hypothyroidism (3%). To mention is one case of jaundice after twenty days of Th 100 mg/day. Contrary to the previous two cases reported in the literature, it was not associated to an increase of transaminases, but only to a marked and irreversible rise in the bilirubine level. Leukopenia occurred in the 36% of patients, but it was related to Melph association rather than to Th dose escalation. PN was the commonest non-hematological side effect of Th in 400 mg/day whereas those who received Th MDD ≤ 150 mg/day developed PN after a median of 12 months whereas those who received Th MDD >150 mg/day developed PN after a median of 23 months.
they significantly hasten peripheral neuropathy. On the contrary, response rates (RR) was not influenced by Th MDD; univariate analysis showed that RR was significantly higher in patients with β2MG <3 mg/L, Hb < 10.5 g/l, prior therapy ≥ 2 regimens and disease history ≤ 3 years. Multivariate analysis showed that the only factor which is associated to a higher RR is the combination therapy Th-Melph. In summary, our results suggest to use of low dose Th (i.e. ≤ 150 mg/day) in association with chemotherapy, since higher doses do not improve the response rate in MM patients whereas they significantly hasten peripheral neuropathy.

PO081
ASYMPTOMATIC IGM MONOCLONAL GAMMOPATHY: CLINICAL EVOLUTION AND PROGNOSTIC FACTORS IN 300 PATIENTS
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Asymptomatic clonal macroglobulinemia (ACM) is currently classified as an IgM MGUS or indolent Waldenström’s macroglobulinemia (WM), but its clinical relevance and propensity to evolve into lymphoid neoplasms is not well defined. We retrospectively evaluated 300 patients with primary and non-requireing treatment ACM in order to identify the clinico-pathological features relating to its evolution into a symptomatic lymphoid neoplasm requiring treatment and create a prognostic score capable of distinguishing patient subgroups with different prognoses. The main characteristics at diagnosis are shown in the table.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of evaluated pts</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (in years) (mean ± SD)</td>
<td>300</td>
<td>63± 11</td>
</tr>
<tr>
<td>M/F (ratio)</td>
<td>300</td>
<td>190:114</td>
</tr>
<tr>
<td>Serum β2 microglobulin mg/mL (median, min-max)</td>
<td>300</td>
<td>1.3 (0.13-3.0)</td>
</tr>
<tr>
<td>β2 microglobulin mg/mL (median, min-max)</td>
<td>300</td>
<td>13.8 (13.1-17.9)</td>
</tr>
<tr>
<td>Peripheral lymphocytes &gt;10×10^9/L (median, min-max)</td>
<td>300</td>
<td>2 (0.4-3.6)</td>
</tr>
<tr>
<td>Platelets (PLT) ×10^9/L (median, min-max)</td>
<td>300</td>
<td>238 (104-627)</td>
</tr>
<tr>
<td>Serum LDH U/l (median, min-max)</td>
<td>187</td>
<td>504 (104-1396)</td>
</tr>
<tr>
<td>Serum β2 microglobulin mg/mL (median, min-max)</td>
<td>132</td>
<td>2.55 (1025-7962)</td>
</tr>
<tr>
<td>No. of pts. with detectable Bence Jones proteinuria (%)</td>
<td>296</td>
<td>21 (296)</td>
</tr>
<tr>
<td>No. of pts. with one serum polyclonal (Ig) reduction (%)</td>
<td>258</td>
<td>34 (250)</td>
</tr>
<tr>
<td>Bone marrow lymphoma cells% (median, min-max)</td>
<td>237</td>
<td>13 (92)</td>
</tr>
</tbody>
</table>

After a median follow-up of 55 months (6-221), 43/300 patients (14.7%) required chemotherapy for symptomatic WM (70%), NHL (18.5%), amyloidosis (7%), or peripheral neuropathy (4.5%). Five- and 10-year overall survival was 98% and 90%, and evolution-free-survival 91% and 78%. The features correlating with evolution to overt lymphoproliferative disease were: serum clonal and serum nephelometric IgM concentration (p<0.0001), Bence Jones proteinuria (p<0.0001), hemoglobin (p<0.0001), nephelometric IgG concentration (p=0.0259), albumin (p=0.0257) and male gender (p=0.04). The evolution-related variables at multivariate analysis were: serum IgM concentration, hemoglobin and male sex. By attributing arbitrary scores to the three prognostic variables (male=2, female=0; Hb <12 g/dL=2, 12-15=1, >15=0; s-clonal IgM <0.7 g/dL=0, 0.7-1.5=1, >1.5=2), we developed a simple prognostic index that separated the patients into three subgroups with different prognoses. The main characteristics at diagnosis are shown in the table.

Several reports indicate the presence of thrombomeg- morrhagic complications in patients with MM. These complications have been generally attributed to the presence of the paraprotein interfering with the normal mechanism of the coagulations system. Aim of this study was to investigate coagulation and thrombophilic parameters in newly diagnosed patients with a monoclonal gammopathy observed during a period of 1 year. From 04/1999 to 03/2000, 167 consecutive patients with newly diagnosed monoclonal gammopathy, observed at the Hematology section of our Department, were enrolled in this study. There were 76 males and 94 females and median age was 67.6 (range 28.1-80.6) yrs. Of these 16 patients, 100 had a monoclonal gammopathy of undetermined significance (MGUS), 62 had multiple myeloma (MM) and 5 Waldenström macroglobulinemia (WM). The coagulation and thrombophilic parameters studied were: PT, aPTT, Fibrinogen, KCT, dRVVT, aPCR, PC and ATIII. The results were compared to those obtained in 62 normal matched controls. Among the MM patients there were a significantly higher number of patients with prolonged PT (p=0.009) and aPTT (p=0.02) than in controls. A pathologically reduced ATIII was observed in 11 patients with MM, in 6 MGUS (p=0.01) and in 1 controls (p=0.01). PC was
PO083
THE USE OF FDG-PET, 99MTC-MIBI SCANNING AND MRI IN MYELOMA PATIENTS: COMPARISON WITH STANDARD SKELETAL X-RAYS AND THE PRESENCE OF BONE PAIN
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Aim: To evaluate the usefulness of total body FDG-PET, Tc99 sesta-MIBI scanning and vertebral MRI versus standard X-ray imaging in determining the extension of bone disease in patients with multiple myeloma (MM). Between March 2002 and May 2003, we enrolled 27 patients (18 male and 9 female): 26 with MM and one with a solitary plasmacytoma. All of the MM patients had measurable serum paraprotein levels. The patients were evaluated before any therapy: 16 had bone lesions at standard radiographic imaging; all 27 underwent PET examination; 23 underwent MIBI scanning and 24 vertebral MRI. Vertebral MRI was performed to detect minimal pre-radiographic alterations in vertebral imaging and verify the FDG-PET results. Results: Radiograms showed that 15/27 patients had skeletal lesions, all of whom had corresponding bone pain; eight were also PET and MRI positive. Three of the 15 patients had isolated vertebral collapse(s) and showed no signs of focal PET positivity, but one had diffuse bone marrow uptake; all three had positive MRI scans. Three PET-positive patients did not undergo vertebral MRI because of technical problems. One patient (1/15) had isolated humeral lesion with focal PET uptake. Vertebral MRI was normal. One patient (1/27) was asymptomatic, but X-ray revealed a humeral lesion and MRI revealed various local and vertebral abnormalities; the PET result was negative. Eleven of the 27 patients had negative X-rays and were free of bone pain. All of the other examinations were negative in four cases; the remaining seven had positive PET scans (six with concomitant positive spinal MRIs). In one patient, local vertebral radiotherapy was given on the basis of the MRI and PET results. Of the 23 patients who underwent total body Tc99-MIBI scans, five showed localised positivity corresponding to the X-ray lesions; three had diffuse humeral and/or femoral uptake and 15 negative results. MIBI positivity always corresponded to PET positivity with the same pattern. Nine of the 15 MIBI-negative patients were PET positive. Conclusions: 1) In MM pts with bone pain, positive X-ray results and a clear indication for treatment on the basis of current hematologic criteria, PET does not influence medical decision making; it might be useful in monitoring skeletal lesions in responding patients but, in cases of vertebral collapse, the results may be negative even in the presence of active disease. 2) PET imaging may be useful in selected asymptomatic patients with negative X-rays as a means of discovering occult skeletal disease; in terms of the evaluation of the spinal column, MRI seems to be more sensitive. 3) Regardless of the radiographic results, diffuse bone FDG-PET positivity should be assessed as a means of evaluating the amount and activity of the tumour burden. 4) MIBI scans are not qualitatively useful, at least in our hands.
P0085
HUMAN MYELOMA CELLS EXPRESS INTERLEUKIN-7 (IL-7) mRNA AND SECRETE IL-7 IN PRESENCE OF IL-6: ROLE OF IL-7 IN MULTIPLE MYELOMA PHYSIOPATHOLOGY
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Interleukin-7 (IL-7) is a critical cytokine involved in T and B lymphopoiesis. The potential role of IL-7 in multiple myeloma (MM) has been investigated in this study. First we tested the IL-7 and IL-7 receptor (IL-7R), CD127, expression in human myeloma cell lines (HMCL) and in purified CD138+ myeloma cells obtained from MM at the diagnosis. We found that RPMI-8226, U266, OPM-2, XG-6 and fresh MM cells were positive for IL-7 mRNA. On the other hand IL-7R mRNA was not expressed in any HMCL tested while the EBV positive cell line ARH-77 was positive for IL-7R. Using an ELISA assay IL-7 was detected in the supernatant of HMCL, in contrast IL-7 was undetectable in conditioned medium of mononuclear cells or normal CD19+ B cells or B leukemic cell line REH. IL-7 levels were significantly up-regulated when RPMI-8226, U266, XG-6 were cultured in presence of IL-6 (20-50 ng/mL) while IL-6 did not induce IL-7 production in normal B cells and REH as well as in EBV positive cells and in B cells obtained from patients with acute lymphoblastic leukemia, previously evaluated for CD126 expression by flow cytometry. A stimulatory effect of IL-7 on ARH-77 proliferation was found (+12% ±p<0.05).

In addition IL-7 induced the production of the critical osteoclastogenetic factor RANKL by T lymphocytes and blocking anti-IL-7 Ab inhibited the stimulatory effect of HMCL on RANKL production by T lymphocytes in tranwell co-culture system. BM stromal cells was also found to be positive for IL-7R and blocking anti-IL-7 Ab reduced the up-regulation of IL-6 induced by myeloma cells in a co-culture system. Following we tested IL-7 levels in MM patients. We found that IL-7 serum levels were significantly higher in MM patients in comparison to healthy subjects (median: 12.15 pg/mL; range: 2.41-29.5 pg/mL vs. 1.91 pg/mL; range: 0-3.43 pg/mL; p<0.05). Similarly, IL-7 levels in BM plasma were significantly increased in MM patients in comparison with normal subjects (median: 8.67 pg/mL; range: 2.68-36.8 pg/mL vs. 0.40 pg/mL; range: 0-0.46 pg/mL; p<0.05). In conclusion our results indicate that myeloma cells express IL-7 and that IL-6 stimulate IL-7 secretion suggesting a vicious loop between IL-6 and IL-7. Furthermore the high IL-7 levels, found in MM patients support the potential involvement of IL-7 in MM physiopathology.

P0086
HUMAN MYELOMA CELLS PRODUCE ANGIOPOIETIN-1: POTENTIAL RELATIONSHIP WITH MYELOMA-INDUCED ANGIOGENESIS
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Multiple myeloma (MM) patients have an increased bone marrow (BM) angiogenesis, however the proangiogenic properties of myeloma cells and the mechanisms of MM-induced angiogenesis are not completely clarified. The angiopoietin system has been identified as critical in the regulation of vessel formation. In this study we have demonstrated that angiopoietin-1 (Ang-1), but not its antagonist Ang-2, was expressed by several human myeloma cell lines (HMCLs) at both mRNA and protein level by RT-PCR and western blot analysis, respectively. Immunohistochemistry and immunoprecipitation confirmed that HMCL produced and secrete Ang-1 but not Ang-2. In a transwell co-culture system, we
observed that myeloma cells up-regulated the ang-1 receptor Tie2 in human BM endothelial cells. Moreover, in an experimental model of angiogenesis the conditioned medium of HMCLs significantly stimulated vessel formation as compared to control or to VEGF treatment. The presence of anti-Tie2 blocking Ab completely blunted the pro-angiogenetic effect of XG-6. Our in vitro results were supported by the in vivo finding of Ang-1 but not Ang-2 mRNA and protein expression in purified MM cells obtained from about 47% of 23 patients analysed. BM angiogenesis was evaluated in bone biopsies from 15 out of 23 patients. The number of microvessels per field was higher in Ang-1 positive patients in comparison with Ang-1 negative ones (mean±SE: 6.23±0.2 vs. 2.94±0.1, median: 6.21 vs. 2.79; p=0.001.) and the microvascular density was significantly increased (32.98±1.7 vs. 14.55±1.3, median: 34.69 vs. 13.04; p<0.01; capillaries: 26.73±1.3 vs. 10.42±0.8; median: 24.06 vs. 9.04; p=0.01, small venules: 9.56±0.5 vs. 4.14±0.5, median: 10.60 vs. 3.65; p<0.01). Furthermore a significant positive correlation between Ang-1 expression and MVD was found in our cohort of patients (Pearson χ²: p=0.036, Cochran's Linear Trend: p=0.01). In conclusion our data indicate that myeloma cells produced Ang-1 that it is involved in MM-induced angiogenesis.

PO087
MYELOMA CELL RECOGNITION BY NATURAL KILLER LYMPHOCYTES

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NK cells are potent cytotoxic lymphocytes, which impair the growth of several tumor cell lines in vitro; however, the in vivo relevancy of human NK cell anti-tumor activity remains to be defined. MHC class I are the main regulatory molecules for both CTL and NK cells. However, MHC class I expression leads to opposite effects in the two lymphocyte subsets. T cell killing is strictly dependent by MHC class I recognition on target cell surface, while NK cell cytotoxicity is prevented by MHC class I inhibitory receptors engagement. Loss of MHC class I is believed to be one of the major escape strategy to immune surveillance in solid tumors. On the other hand, stress inducible molecules (MICA, MIICB, ULBPs) are expressed during the neoplastic transformation and may activate both immune effec-
tors mechanism. In our study we have addressed the following questions: 1) How is the tumoral mass edited by host immune system in myeloma disease? 2) At which stage of the disease are MIC and ULBP expressed? 3) Are NK cells able to recognize freshly established autologous myeloma cells as target? 4) Which molecules are involved in the regulation of autologous and allogeneic NK cells reactivity against myeloma? We have addressed the above questions using an experimental myeloma cell system composed by bone marrow (BM), peripheral blood (PB) and pleural effusion (PE) and tumor autologous NK cells obtained ex vivo. Our data demonstrate that the bone marrow derived myeloma cell lines are susceptible to NK cell cytoxicity. This finding was confirmed with ex vivo autologous cytotoxic experiments. However, all pleural effusion cell lines were resistant to NK cell recognition. The lack of NK recognition of cells derived from myeloma in advanced state appears to be MHC class I dependent. MIC and ULBPs expression data will be presented and discussed as well.

PO088
CLINICAL AND ELECTROPHYSIOLOGICAL EVALUATION OF NEUROPATHIES DEVELOPED DURING THALIDOMIDE TREATMENT FOR MULTIPLE MYELOMA

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Thalidomide is a glutamic acid derivative with anti-angiogenic and immunomodulating properties, that has recently proven effective as a single agent in relapsed/refractory multiple myeloma (MM). Furthermore, low dose thalidomide (Thal) and dexamethasone (Dex) combination is effective in at least 50% of pts resistant to chemotherapy. Peripheral neuropathy, mainly axonal and sensory, has emerged as a principal dose-limiting side effect of long-term Thal treatment. In order to further evaluate drug-induced neurotoxicity we performed a prospective study based on clinical and electrophysiological follow-up of MM pts treated with Thal. Twenty-eight relapsed/refractory pts (20 men and 8 women), median age 64 years (range 54-80) were enrolled in an open-label trial of oral low-dose thalidomide (100-200 mg day) plus dexamethasone (40 mg, day 1-4, every month), and underwent neurologic examination and nerve conduction study (NCS) at time 0 (before treatment) and every three months during therapy. Neurologic examination included grading of symptoms, signs and NCS according to the total neuropathy score (TNS). NCS also comprised recording of sensory nerve potentials (SNAPs) and conduction velocity (CV) from median, radial and sural nerves, and motor
nerve action potentials (MNAPs), CV and F waves from median, ulnar, tibial and peroneal nerves. Before treatment three patients showed signs of axonal demyelinating neuropathy, and one patient a pure axonal neuropathy. None of the 28 and 11 pts evaluated at 3 and 6 months, respectively, showed clinical or electrophysiological changes. Six pts underwent the nine-month and 3 pts the twelve-month control; all of them showed new mild sensory neuropathy or aggravation of pre-existing alterations, requiring Thal discontinuation in 2 cases. Only one patient performed a control at 15 and 18 months, showing a mild worsening that did not require therapy withdrawal. These preliminary results suggest that drug-induced neuropathy represent a complication of low-dose Thal plus Dex treatment in MM pts. Further studies are warranted to better evaluate long-term neurotoxicity of this association.

PO009
THALIDOMIDE IN COMBINATION WITH DEXAMETHASONE AND CYCLOPHOSPHAMIDE FOR RELAPSED/REFRACTORY MULTIPLE MYELOMA
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Introduction: The antmyeloma effect of thalidomide (Thal) alone has been demonstrated in several clinical trials. Recent data indicate that Thal can increase the therapeutic effect of chemotherapy and might be able to overcome drug resistance. Response rates of 25% with Thal used as a single agent, and up to 75%, when used in combination with other agents, have been observed. The optimal schedule, dosage and association with other drugs is still not established. AIM: to evaluate the feasibility and efficacy of Thal in combination with Dexamethasone (Dex) and cyclophosphamide (CTX) for relapsed/refractory myeloma (MM). Patients and Methods: Between October 2001 and May 2003 sixteen patients (pts) (12 M / 4 F) with relapsed/refractory MM were enrolled in an open-label trial of oral low dose Thal (100-200 mg/day) plus Dex (40 mg, day 1-4, every month) and cyclophosphamide (500 mg iv/week). Main pre-treatment characteristics were the following: median age 68 years (range 54-76); median B2M 3.3 mg/L (range 1.2-14.2); median bone marrow plasma cell infiltration 30% (range 4-80). Median time from MM diagnosis to treatment was 60 months (range 6-132). All pts were heavily pre-treated. In particular, 12 pts received a median of 3 pre-treatment chemotherapy regimens (range 1-5) and 4 underwent autologous stem cell transplantation. In addition 12 pts showed disease progression during previous treatment with Thal alone or combined with Dex. The EBM/IBM TR/ABM TR criteria were used for definition of response, while toxicity was graded according to WHO criteria. Results: With a median follow-up of 4.5 months (1-12), 15 pts were evaluable for response (1 too early): 11 (73.3%) responded to this association therapy, including 8 with a partial response and 3 with a minimal response; 3 pts (20%) showed no change and 2 (13.3%) progressive disease. At present, 12 pts are alive and 11 are still maintaining the response from a median of 4 months (1-10); 4 pts died due to disease progression, including one who progressed after a 5 months response. Adverse effects were moderate (grade <=2). No cases of thrombocytopenia grade >=2 were observed, while 2 pts experienced neutropenia requiring supportive treatment with G-CSF. Other side effects included grade <=2 constipation (30%), somnolence (35%) or dizziness (10%). No cases of deep venous thrombosis were observed. Conclusions: These results show that the Thal plus Dex and CTX combination is active and feasible in heavily pre-treated multiple myeloma pts, including those relapsing after Thal+Dex therapy. Further studies and a longer follow-up are warranted to evaluate the duration of favorable responses, the effect on survival and possible long-term side effects.

PO090
LOW DOSE THALIDOMIDE FOR TREATMENT OF ADVANCED MULTIPLE MYELOMA
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Thalidomide has emerged as a new class of active agents in MM both as monotherapy and in combinations with other agents, particularly dexamethasone. To date, however, the effect of dose on response, tolerability and biologic response remains poorly understood. To investigate the effect of low dose thalidomide on response rate and toxicity, twenty patients with pretreated MM were enrolled. The characteristics of this group included 12 pts with IgG (10 K and 2 L), 6 pts with IgA (4 L and 2 K) and 1 micro K types of MM. The median age was 69 years old (range 55 - 82), there were 11 males and 9 females. Six pts were at stage III, 11 pts at stage II and 3 pts at stage I of the disease. At the time of thalidomide treatment 15 pts presented relapsed or refractory disease and 15 pts presented stable disease. Previous therapy included tandem autologous PBPC transplant in 8 pts, standard melphalan+prednisone in 10 pts and other chemotherapies in 2 pts. Thalidomide was administered at 100 mg/d orally and maintained at this dosage for at least 3 months. Responding pts received maintenance dose of 50 mg/d, while no responding pts withdrawn the drugs. Treatment was continued until disease progression or serious toxicity. In 12 pts dexamethasone was
added at a dose of 40 mg every week and in 3 pts oral melphalan was also administered, the 5 pts with stable disease received thalidomide alone. The treatment was performed on outpatient basis. Therapy was generally well tolerated. Most of adverse effects were recorded as WHO grade I and II and included constipation (6), sedation (6), edema (5), dizziness (4), tremor (3), peripheral neuropathy (2), bradycardia (1) and dispnoea (1). One patient required definitive drug discontinuation due to neurologic toxicity after 12 months, and 1 patient had temporary discontinuation because of deep venous thrombosis. One patient withdrawn thalidomide within one month and was not evaluable for the response. After a median follow-up of nine months (range 3-15); four patients (21%) had a complete response (>90% reduction in M protein), eight patients (42%) had a partial response (six >50% and two >25% reduction in M protein) and four patients (21%) had stable disease. Three patients (16%) had progressive disease while on therapy. Our study suggests that low dose thalidomide is well tolerated and active in advanced MM with an overall response rate of about 80%. Longer follow-up in a larger population will be needed to validate the results.

PO091
LOW-DOSE THALIDOMIDE AND DEXAMETHASONE IMPROVES SURVIVAL IN MULTIPLE MYELOMA PATIENTS
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Oral melphalan and prednisone has been the standard treatment of multiple myeloma for more than 30 years. Clinical outcome has been improved by high-dose chemotherapy and autologous stem cell transplantation but relapses constantly occur and resistance to chemotherapy remain the major cause of death. The search for new/old drug has led to the selection of thalidomide, this drug is effective in refractory and recurrent myeloma. We evaluated the efficacy of low dose thalidomide (THAL) plus dexamethasone (DEX) in patients with relapsed or refractory multiple myeloma. Between July 1999 and October 2001 we treated 120 relapsed and refractory multiple myeloma patients (median age 63), with THAL 100 mg/day (continuous) and DEX 40 mg (days 1-4 of each month). Their clinical outcome was compared to a control group of 120 relapsed and refractory multiple myeloma patients (median age 62) treated with conventional chemotherapy (CC). Both groups have similar clinical characteristics. Results of patients receiving THAL-DEX or CC after one line of chemotherapy only (early stages of disease) were showed separately from those of patients treated after two or more lines of chemotherapy (late stages of disease). THAL-DEX regimen significantly improved outcome in patients treated after one line of chemotherapy only. Myeloma protein reduction 50%-100% was observed in 56% of the THAL-DEX group and in 46% of the CC group. The probability of progression-free survival (PFS) for 3 years was 38% in the THAL-DEX group and 6% in the CC group (p=0.0024). The estimated survival for 3 years was 60% in THAL-DEX group and 26% in CC group (p=0.0016). Clinical outcome was similar in patients receiving THAL-DEX or CC after two or more line of chemotherapy, Myeloma protein reduction 50%-100% was observed in 46% of the THAL-DEX group and in 42% of the CC group. The probability of PFS for 3 years was 11% in the THAL-DEX group and 3% in the CC group (p=0.23). The estimated survival for 3 years was 22% in THAL-DEX group and 12% in CC group (p=0.45). Most adverse effects were recorded as WHO grade I, 12% of patients displayed a grade II toxicity and 4% grade III. Constipation was relatively frequent (17% of patients). Sedation was recorded in 13% of patients, and 7% showed confusion. Tingling and numbness were observed in 11% of patients as grade I, in 8% as grade II. Tremors and incoordinations were present in 6% of patients and were generally mild. Discontinuation was required in 18% of patients, mainly due to neurologic toxicity (11%). THAL-DEX is superior to CC in the earlier phases of disease. THAL-DEX and CC are equivalent in the more advanced stages of disease. Thalidomide is well tolerated and this regimen is not myelotoxic, postpones the delivery of chemotherapy, and therefore the development of resistant disease.

PO092
BASIC FIBROBLAST GROWTH FACTOR (BFGF) PRODUCTION AND ROLE IN MULTIPLE MYELOMA PATIENTS
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Basic FGF (bFGF) is a growth factor with pro-angiogenic properties. Elevated bone marrow (BM) and peripheral serum bFGF levels have been reported in multiple myeloma (MM) patients; however, the source
of bFGF in MM patients is not completely elucidated. In order to better clarify this issue we wish to make our evidence. Using RT-PCR, we found that human myeloma cell lines (HMCLs) XG-6, RPMI-8226, OPM-2 as well as EBV positive cell line ARH-77 did not express bFGF mRNA while U266 was positive and XG-1 expressed bFGF at low intensity. Similarly, we failed to detect bFGF either in HMCL lysates by western blot analysis or in HMCLs (10⁶/mL) conditioned media by ELISA both in the presence and absence of IL-6 (20 ng/mL) with the exception of U266 and XG-1. Consistently, we showed that blocking anti-bFGF Ab failed to inhibit HMCL-induced angiogenesis in an in vitro system. Purified CD138+ MM cells (purity > 90%) isolated by an immunomagnetic method were positive for bFGF mRNA expression in 11 out of 35 patients with newly diagnosed MM in stage I–III (median age: 64, range: 33–88; median plasmocytosis: 35%, range: 12–95%). In contrast BM stromal cells (BM SC) obtained from all patients were positive for bFGF mRNA. bFGF protein has been found in plasma cell lysates in 8 out of 30 patients tested. Consistently, bFGF levels were detected by ELISA in conditioned media of purified MM cells (10⁶/mL) in 7 out of 28 patients. Furthermore a nuclear bFGF immunostaining with low cytoplasmatic positivity has been found in bone marrow myeloma cells of 3 out of 21 patients. Any correlation has been found between bFGF expression by MM cells and BM plasmocytosis (Pearson Chi-square: p=0.35) or the presence of osteolytic lesions. The differences in the microvascular density (MVD) and in the number of microvessels per field, between bFGF mRNA positive and negative MM patients did not reach a statistical significance (MVD±SE: 36±4 vs. 24±3.2, number of microvessels±SE: 7.4±5 vs. 3.59±0.5; Mann-Whitney test: p=0.19 and p=0.16, respectively). In conclusion, our data indicate that bFGF is rarely produced directly by MM cells, suggesting that bFGF is not the major pro-angiogenetic factor produced by myeloma cells even if its production could be involved at least in part in the MM-induced angiogenesis.

P0093
ADDITION OF CYCLOPHOSPHAMIDE TO THE COMBINATION OF THALIDOMIDE AND DEXAMETHASONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA
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The combination of thalidomide and dexamethasone has been shown to be more active than the single drugs in the treatment of multiple myeloma. To further enhance the efficacy of this combination of drugs, we added Cyclophosphamide, another agent whose activity against myeloma has been proven. Forty patients with a median age of 70 years, not eligible for a transplantation procedure, were included in this trial. Seven were relapsing and 33 were resistant or progressing after a median number of two previous treatments. The time elapsed between diagnosis and entering this study was 23 months (range 2–70). At time of starting this protocol, five patients were in stage I, 3 in stage II and 32 in stage III. The median Hb value was 10.2 g/dL (range 6.6–13.7), median WBC count 2.9 ×10⁹/L (range 0.6–7.2), median PLT count 159×10⁹/L (range 48–347). DEX was given at the fixed dose of 40 mg/day for 4 days every month, while THAL at 200 mg every evening and CTX 100 mg every morning continuously. Response to treatment was evaluated by the percentage of reduction of the monoclonal component (MC). Two patients were not evaluable: one died for progression of disease a few days after starting treatment, and the other refused treatment because developed a skin reaction to THAL two days after starting treatment. Among the remaining 38 patients, 35 (91%) had some degree of response while 3 (8%) were considered as non responsive. In particular, eight patients (21%) had a reduction of MC < 50%, 8 patients (21%) < 75%, and 19 patients (50%) > 75%. Of the latter, 8 (21%) achieved a complete response (100% reduction of MC). In these latter patients bone marrow evaluation showed the disappearance of plasma cells infiltration and in three of them a negativity of IF was achieved. Median time between start of treatment and evaluation of response was 7 months (range 2–26). No differences of response were observed among different Ig isotypes. After a median follow up time of 13 months, 12 of the responding patients (32%) have experienced a relapse after a median time of 5 months (range 2–19) from evaluation of response. Side effects of the combination included constipation, nausea, somnolence, asthenia, fever diarrhoea. Eight patients did not received DEXA because of diabetes and in 6 additional patients it was discontinued because of side effects. Nine patients discontinued treatment with THAL (five of them definitely) because of skin rash, peripheral neuropathy (4 patients), poor compliance in a patient affected by Alzheimer’s disease, pneumonia, FUO, dizziness. CTX was temporarily discontinued in 23 patients because of leukopenia (20 patients), nausea, hematuria, or Alzheimer’s disease. In conclusion, the combination of THAL-CTX-DEX seems to be a very active and a well tolerated scheme against refractory myeloma. The high rate of discontinuation of CTX should be evaluated in the light of the poor bone marrow reserve of patients included in this study (median WBC 2.9). Therefore, the role of CTX in this combination should be investigated in less heavily pretreated patients.
CONCLUSION

Most of plasma cell malignancies show clonal plasma cells in bone marrow aspirates and serum immunofixation and <5% PC on BM biopsy) was 26% and 33% after the first and the second course of myeloablative treatment, respectively, in patients reinfused with selected cells, in comparison with 17% after a single ASCT and 25% after a double ASCT in patients who received an unpurged graft (p=0.8). Median follow-up is 43 months in both arms. So far there is no significant difference in progression-free survival (PFS) and overall survival (OS). Probability of PFS at 60 months after a single ASCT was 26% in the selected group and 26.5% in the unselected one (p=0.8) and OS was 71% and 35% respectively (p= 0.4). Moreover, no significant difference was found when comparing PFS and OS curves after double transplantation between the two groups. We conclude that reinfusion of CD34+ selected stem cells did not delay hematologic engraftment, lowered levels of CD4+ lymphocytes within 1 year post-transplant without excess of late infections, tended to produce a higher rate of complete responses but had no significant clinical benefit for PFS either after single or double transplantation, in comparison with reinfusion of unselected autologous stem cells.

Rawstron et al (Blood 2002) have recently described a flow cytometric procedure for disease monitoring in myeloma. Malignant plasma cells were enumerated as CD56+CD38+CD138+ cells. Patients with only CD56− plasma cells had a low risk of disease progression. Patients with CD56+ plasma cells had a significantly higher risk of early disease progression. In addition to CD56 expression, the same group has proposed in the past CD19, CD45 and CD126 expression as markers of plasma cell malignancy. Sahara et al (Br J Haematol 2003), however, have reported that about 20% of myeloma patients, including most of plasmablastic cases, lack CD56 expression on malignant plasma cells. Thus, we asked what marker of plasma cell malignancy, among clonality (ie intracellular kappa or lambda chain expression), CD19, CD20, CD45, CD56 or CD126, better correlated with immunohistochemistry (IHC) myeloma pathology evaluation in bone marrow biopsies and with disease progression. We evaluated 172 consecutive marrow aspirates from 81 patients. Overall, 68 patients had myeloma, 5 had solitary plasmacytoma and 8 MGUS. Plasma cells were depicted as CD38−
CD138+ cells, gated, and evaluated for κ, λ, CD19, CD20, CD45, CD56 or CD126 expression. In 120/172 cases (70%), plasma cells were found to be monoclonal, ie with a κ/λ (or vice versa) ratio >5. In 8/172 (4%) cases the κ/λ ratio was borderline (3.0-4.9), the remaining 44/172 cases (26%) were polyclonal (ratio <3.0). The concordance between flow cytometric clonality and IHC in defining a sample as positive for myeloma was 81%, and a very strong positive correlation (r=0.803, p=0.00001) was found between the frequency of malignant plasma cells evaluated by flow cytometry and the frequency of malignant plasma cells evaluated by IHC. Overall, in 39/120 cases (32.5%), monoclonal plasma cells lacked CD56 expression. In grade I, II and III myeloma patients, monoclonal plasma cells lacked CD56 expression in 33, 25 and 37% of cases, respectively. The presence or the absence of CD56 expression was consistent over time, because it was not modified at the second or third evaluation in 89% of patients. In 28% of cases, polyclonal plasma cells had CD56 expression on more than 30% of cells. Using CD56 as a flow cytometric marker of plasma cell malignancy, the concordance between flow cytometry and IHC in defining a sample as positive was 64%, ie significantly inferior than clonality (p=0.0002). In 93, 85, 47 and 86% of cases, monoclonal plasma cells lacked CD19, CD20, CD45 or CD126 expression, respectively. Using CD19, CD20, CD45 or CD126 expression as markers of malignancy, concordance between flow cytometry and IHC was 20, 34, 41 and 31%, respectively (p<0.00001 vs clonality). To investigate the prognostic potential of plasma cell clonality evaluation, we selected 11 elderly myeloma patients treated with 6 cycles of VAD and 14 myeloma patients treated with high-dose chemotherapy. In all patients receiving VAD, monoclonal plasma cells were still present at the end of the therapy. In 7 out of 14 patients receiving high-dose chemotherapy plasma cells were polyclonal 3-4 months after therapy. Among patients who had polyclonal plasma cells after high-dose chemotherapy, 6 out of 7 were in remission after a median follow up of 17 months. Among the 7 patients who had monoclonal plasma cells found in the marrow after high-dose chemotherapy, only 2 were in remission at the end of the follow-up (p=0.01). In conclusion, clonality appears to be superior to other flow cytometric markers of disease monitoring in myeloma. We are prospectively monitoring our patient population to gain more insight into the prognostic potential of this procedure.

Bone involvement represents a common feature of multiple myeloma (MM). Evaluation of biochemical markers of bone turnover could offer a dynamic perspective of the effects of a given therapy on bone metabolism. In patients who were enrolled in the Bologna 2002 phase II clinical trial and were treated at our center, markers of bone resorption (urinary NTX, PYR and DPYR and serum crosslaps) and bone formation (bone alkaline phosphatase-BAP and osteocalcin) were routinely evaluated at diagnosis and at various time points during therapy. By study design, all patients received four months of combined thalidomide (100 mg/d for two weeks and 200 mg/d thereafter) and dexamethasone (40 mg/d, on d 1-4, 9-12, 17-20/28 d on odd cycles and on d 1-4/28 d on even cycles) therapy (THAL-DEX) as induction of remission before peripheral blood stem cell (PBSC) collection with high-dose cyclophosphamide and subsequent double autotransplants upon treatment with melphalan 200mg/sqm. Zoledronic acid (ZOLE acid) was administered at 4 mg/28 d for at least 9 months. Data from 28 patients (15M, 13F, median age = 53 years) have been collected so far. At diagnosis, all bone resorption markers were increased in more than half of the patients, while BAP and osteocalcin were decreased in 29% and 18% of the patients, respectively. Both urinary NTX (p=0.039) and serum crosslaps (p=0.000) were positively correlated with the extent of skeletal involvement, graded according to the number and the size of osteolytic bone lesions assessed in whole skeleton X-ray. After 4 months of therapy with THAL-DEX and ZOLE acid a significant decrease in mean urinary NTX (58.6±9.5SE nmol/mmol crea vs 21.2±5.1SE, p=0.003) and serum crosslaps (5600±1324SE pmol/L vs 1985±572SE) was observed. Other resorption markers were also reduced, though not significantly. A slight decrease in bone formation markers was also detected, possibly as a result of DEX therapy; however, this finding needs to be confirmed at a subsequent analysis performed at the end of the whole treatment program. It is concluded that among all the markers of bone turnover, serum crosslaps and urinary NTX are the ones most strictly related to actual bone resorption and to the extent of bone involvement, as evaluated at X-ray survey. Combined THAL-DEX and ZOLE acid administered as primary therapy for patients with newly diagnosed and symptomatic MM seem to be highly effective in reducing bone turnover.
po097
THALIDOMIDE, DEXAMETHASONE AND ZOLEDRONATE FOR THE TREATMENT OF MYELOMA PATIENTS RELAPSED AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: COMPARISON WITH OTHER SALVAGE THERAPIES


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Thalidomide induces significant responses in about 30% of patients with pre-treated multiple myeloma (MM). Higher rates have been reported by combining thalidomide and dexamethasone, another agent with well known efficacy in MM, due to the possible synergistic activity of these two drugs. Zoledronate is a new generation bisphosphonate, which has been demonstrated to be active on bone disease in MM, but which also seems to exert anti-myeloma effects. Based on these data, we administered, on an out-patient basis, a combination of thalidomide (200 mg/d), dexamethasone (40 mg for 4 days every 4 weeks) and zoledronate (4 mg every 4 weeks) to 22 MM (mean age 57 years), who had relapsed after frontline AuSCT and who were not suitable for a second transplant. Twenty-one patients received at least 12 weeks of therapy. Somnolence, sedation, oedema, constipation, fluid retention and skin rash were the most relevant side effects observed, occurring in 11 patients. The reduction of thalidomide dose to 50-100 mg/d resolved these adverse events. Two patients required reduction of dexamethasone to 20 mg/d due to hypertension and hyperglicemia. Asymptomatic hypocalcemia was also observed in 6 patients and was corrected by substitutive therapy. No patient developed thrombotic complications, but one patient interrupted early the trial, due to severe pancytopenia. Among the 21 evaluable patients, four stopped the treatment after 12 weeks because of inefficacy. Four additional patients showed progressive disease (in two cases after an initial moderate reduction of M-component). The remaining 13 patients (59%) evidenced a significant response. In particular, a reduction of M-component > 25%, > 50% and >75% was observed in 2, 7 and 3 patients, respectively, while one subject achieved complete remission, with disappearance of the paraprotein at immunofixation. After a minimum follow-up of 18 months, median PFS and OS are still not reached. Interestingly, in 3 patients PFS was longer than that observed after AuSCT. These results were comparable with those we obtained in the same setting of patients (n. 15) using single or double AuSCT as salvage therapy, employing a reduced-intensity conditioning regimen (Mel-100), but were significantly better when compared with 13 historical controls treated with conventional salvage chemotherapy without stem cell support (median PFS and OS from starting of salvage therapy: 7 and 14 months, respectively). Our data suggest that the combination of thalidomide, dexamethasone and zoledronate is probably the best currently available salvage treatment for MM patients relapsed after AuSCT, who do not have the possibility to undergo a second transplant procedure.

po098
OCCURRENCE OF HODGKIN’S DISEASE IN MULTIPLE MYELOMA PATIENT. CASE REPORT

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Rare cases of coexisting multiple myeloma (MM) and Hodgkin’s lymphoma (HD) are described. Sometimes patients with HD develop MM after radiotherapy and/or chemotherapy with alkylating agents. In other cases MM patients with HD present monoclonal gammopathy with or without overt MM. We report the case of a 46 year-old male patient with bone pain, lumbar vertebral lytic lesions. Other laboratory findings showed slight anemia (hemoglobin 10.6 g/dL), erythrocyte sedimentation rate of 74 mm/h, C-reactive protein of 61 mg/dL, IgG-λ monoclonal component (2200 mg/dL) and Bence-Jones protein in urine (>1g/24h). Bone marrow biopsy showed a plasma cells infiltrate up to 20%: thus the diagnosis was MM IgG lambda stage III A. Patient received 4 cycles of VAD regimen and monthly bisphosphonate administration, achieving complete medullary remission with persistence of bony lytic lesions at MRI. Subsequently high dose Endoxan and peripheral blood stem cell (PBSC) collection were performed. After PBSC collection patient developed fever and chest x-ray and CT scan revealed right lung basal thickening. Suspecting pneumonia, patient received antibiotic and antifungal treatment without any benefit. A biopsy of lung thickening was performed and pathology revealed scleros nodular Hodgkin disease. Total body CT showed lymphnodal involvement in chest and abdomen and splenic localisation of disease. PET scan showed similar findings. Because disease onset after anthracyclin containing regimen, patient was treated by non cross-reactive therapy (ESHAP for two courses), obtaining complete remission. After patient was submitted to BEAM chemotherapy regimen followed by PBSC rein-

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PO099
PREVALENCE OF SERUM MONOCLONAL PROTEINS IN A BLOOD DONOR POPULATION
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Background: Presence of serum monoclonal proteins (MP) is often reported as an occasional finding in routine protein electrophoresis, particularly after the adoption of more sensitive assays. Low concentration MP may be secondary to a coincident disease, or primitive, and thus classified as monoclonal gammopathy of undetermined significance (MGUS). High concentration MP may indicate a serious disease such as multiple myeloma, Waldenstrom's macroglobulinemia or amyloidosis. Limited information is presently available on prevalence and follow-up of MGUS in a healthy population. Since 2002 we have been performing serum protein electrophoresis in our 17,000 regular blood donors. In this study we evaluated the prevalence of MGUS in our healthy donor population and the distribution of MP accordingly to sex, age, immunoglobulin class and chain type. Materials and methods. All repeat donors (median age 42 years, range 18-68) had an annual automated gel-based electrophoresis (Hydragel b1-b2, Hydrasis LC, Sebia, Issy-les-Moulineaux, France) included in the set of laboratory tests performed at the end of donation. Donors showing MP had immunofixation (Hydragel IFE, Sebia, Issy-les-Moulineaux, France) determined on the same sample and were recalled for diagnostic work-up aimed at ascertaining the cause of MP. Determination of β2-microglobulin, serum electrolytes, creatinine clearance, uric acid, serum calcium, lactate dehydrogenase, liver enzymes, urinary proteins and urine immunoelectrophoresis were performed together with a specialistic medical visit. Donors confirming the presence of an MP <1.5 g/dL were followed-up every 6 months, undergoing the same tests. Donors with MP > 1.5 g/dL were referred to the hematology department for further evaluation. As far as eligibility to donate blood, we decided to exclude temporarily these donors from donating. Results. In 2002 we examined 11,114 donors (7,276 M, 3,838 F). Overall prevalence of MP was 0.89%. No statistically significant difference was found between males and females (0.91% and 0.83%, respectively). The risk of displaying MP progressively increased by ageing, starting from the third decade (p<0.05) (Table 1). Overall, 8 donors displayed more than 1 MP. A significant (p<0.05) higher portion (83%) of donors with MP displayed an MP of IgG class. No significant difference was found between chain types. Median MP concentration (g/dL) and range were 0.2 (0.06-1.12), in particular: IgG 0.27 (0.07-1.12); IgM 0.23 (0.06-0.77); IgA 0.26. None of these donors presented urinary MP. During the follow-up, 6 of 43 donors became negative for CM and none showed an increase of MP concentration. Conclusions. Monoclonal proteins, mainly of the IgG class, have been identified in almost 1% of our healthy blood donors. Our data are not comparable with others in the literature, because our population of eligible donors is a healthy population, by definition. It is worth noting that the risk of displaying MP was significantly higher in subjects over 30 and MP concentrations were particularly low. Longer follow-up of this healthy population is necessary to determine the predictive value of the found abnormalities.

References
consider a therapeutic strategy in which sensitive cases will go straight to Autologous Stem-Cell Transplantation and resistant cases will receive an alternative treatment. Recent data indicate that Thalidomide (Thal) can increase the therapeutic effect of chemotherapy and may be able to overcome drug resistance. However, when Thal is part of a combination that includes Doxorubicin, the incidence of deep venous thrombosis (DVT) increases substantially. We have investigated the activity of Thal (400mg daily) in conjunction with dexamethasone (40mg d. 1-4), Cisplatin (15mg/m² Cl d. 1-4), Cyclophosphamide (400mg/m² d. 1-4), Etoposide (40mg/m² Cl d. 1-4) (DCEP); repeated every 28 days for 4 cycles, in five newly diagnosed MM patients, included in a program of mobilization of PBSC and high-dose treatment, who had shown a resistant or progressive disease after 2 VAD cycles. All patients (pts) had stage III disease (Durie & Salmon). Their mean age was 55 years; PS 1-2. There were 2 female, and 3 males. Three had IgG kappa, one IgG lambda and one IgA κ. Thal was administered for a median of 120 days before mobilization with CTX (7g/m²) and G-CSF (10 mcg/kg) was started 24 hrs after chemotherapy and administered until the last day of leukapheresis. The median of the CD34+ cells collected was 7×10⁶/kg (range 2.8-15.1). Thal plus DCEP obtained a reduction >50% of M-component and plasma cell marrow infiltration in four of the five pts. Thal-DCEP side effects were WHO grade II peripheral neurotoxicity for one patient. No patient developed DVT. Our study indicates that T-DCEP is applicable to pts. with MM eligible for autologous and allogeneic blood stem cell transplantation as a remission inducing treatment and does not alter the release of marrow progenitors into the peripheral blood.

INFECTIONS, QUALITY OF LIFE, SUPPORT THERAPY

PO101 MUCORMYCOSIS IN HEMATOLOGICAL PATIENTS IS A CURABLE INFECTIOUS COMPLICATION?


GIMEM A, Infection Program

Objective: To evaluate the clinical characteristics of patients affected by hematological malignancies who developed mucormycosis and to ascertain the factors which influenced the outcome following mycotic infection. Design: A retrospective study conducted over a 15-years period (1987-2001). Setting: Eighteen Hematology Divisions in tertiary care or university hospital. Patients: The study included 59 patients with hematological malignancies (30 acute myeloid leukemia; 16 acute lymphoid leukemia; 6 lymphoma; 2 hairy cell leukemia and 2 myelodysplastic syndrome; 1 Hodgkin’s disease, 1 multiple myeloma, 1 chronic myeloid leukemia) all with a proven or probable mucormycotic infection. Results: Fever, thoracic pain, dyspnea and cough were the most frequent presenting symptoms of infection. At the onset of the infection, 80% of patients were neutropenic (neutrophil counts < 0.5×10⁹/L) with a median duration of previous neutropenia of 7 days (range 4-30). The most frequent sites of infection were lung (64%) and orbito-sinus-facial (24%); cerebral involvement observed in 19% of cases was always associated with other sites of infection. In vivo diagnosis of mucormycosis was made in only 32 patients (54%). Of the 33 autopsies carried out, 31 was positive. When performed, thoracic and cranial CT scan were the most useful diagnostic investigations. Antifungal treatment was empirically administered in 49 patients (83%); 7 patients underwent a radical surgical debridement (12%). Therapy was successful for only 18 patients (37%). Forty-seven patients died within 3 months after diagnosis of fungal infection: the cause of death was mucormycosis in 41 patients (87%) and progression of hematological disease in 6 patients (13%). At univariate analysis, the factors that correlated with a positive outcome from infection were as follows: male sex, AmB treatment, neutrophil recovery from post-chemotherapy aplasia. At multivariate analysis, the only factor that significantly correlated with recovery from infection was the L-AmB treatment. Conclusion: Mucormycosis is a rare filamentous fungal infection that occurs most frequently in neutropenic patients with acute leukemia. It does not seem to be increased in recent years. Although a reduction of mor-
tality is observed recently, it still remains high. Extensive and aggressive diagnostic and therapeutic procedures are essential in order to improve the prognosis in these patients.

PO102
PEG-INTERFERON a-2B PLUS RIBAVIRIN FOR INITIAL TREATMENT OF CHRONIC HEPATITIS C VIRUS CORRELATED MIXED CRYOglobulinemia: MULTICENTRIC STUDY
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Conventional therapy for chronic HCV is actually based on the employment of combination therapy with peg-interferon (a) plus ribavirin (RBV). There are reports that combination with interferon-a plus ribavirin therapy is efficacious in the treatment of patients with HCV positive mixed cryoglobulinemia (MC), while there are not controlled studies of combination therapy with PEG-IFN plus RBV in patients with HCV positive MC. Aim of the Study: The aim of this study was to evaluate the efficacy and the tolerance of therapy with PEG-IFN + RBV in patients with HCV positive MC. Materials and methods: Fifteen consecutive patients with MC were recruited into the study (8 F / 7 M, median age 52 + 8 yrs). Of these, 80% had type II MC and 20% had type III MC with a median cryocrit of 4.1%. Severe chronic hepatitis was present in 11 cases, while mild chronic hepatitis in 4 cases. All cases were HCV-RNA positive, genotype 1 (53%), or non-1 genotypes (47%). The 15 patients were treated with peg-interferon a-2b at a dose of 1 microg/kg per week plus Ribavirin 800 or 1000 mg per day for 24 weeks and after followed for an additional 24 weeks. Only medium to low-dose steroids were allowed, already administrated at the time of recruitment. Results: At the end of therapy, we observed a reduction of the mean cryocrit level (from 4.12±2.0 to 0.6±0.9% p<0.02), of the mean ALT level (from 141.9±51 to 36±6 U/L p<0.05), and of the purpura score (from 2.0±6 to 0.4±0 p<0.05). Moreover, we observed the complete virologic response in 11 cases (73.3%). Virologic response was greater in patients with genotype non-1 (5 cases) than in patients with genotype 1 (2 cases). In 1 patient with glomerulonephritis, a reduction in proteinuria was observed but at the completion of therapy, the proteinuria returned to pretreatment levels. Four cases (26.6%) obtained no response. Treatment was well tolerated. One case discontinued therapy to 12 weeks for autoimmune thyroiditis. At the end of follow up 7 patients (46.6%) had sustained virological, biochemical and a symptomatic vasculitis response, without detectable cryoglobulins in serum. Conclusions: In patients with MC, PEG-IFN plus RBV therapy was more effective than combination standard (IFN + RBV). Clinical and immunologic response seems to be correlated with the eradication of HCV. PEG-IFN plus RBV may represent a safe and effective alternative to standard immunosoppression in MC.

PO103
NUTRITIONAL SUPPORT AND INFECTIOUS COMPLICATIONS IN HEMATOLOGIC PATIENTS TREATED WITH HIGH DOSE CYTARABINE
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Introduction. Parenteral nutrition is indicated in patients with high dose chemotherapy and radiotherapy gastrointestinal failure. The potential effects of this support (possibly supplemented with glutamine) have been shown to include stimulatory effects on lymphocytes and mucosal cells proliferation in vitro and in animal models. On this basis parenteral nutrition is indicated to prevent bloodstream infection due to translocated colonic bacteria. However, since some randomised controlled studies suggest a correlation between bloodstream infections and central venous catheter (CVC) manipulation, the clinical benefits of parenteral nutrition in hematological patients treated with high dose Ara-C are still controversial. The aim of this study was to perform a retrospective study in order to evaluate the association between parenteral nutrition and infectious complications. Materials and methods. Seventy-two consecutive patients treated with high dose cytarabine for hematological malignancies between October 1999 and June 2003 have been studied. The cumulative cytarabine dose was superior or equal to 8 g/m² per course and the maximum infused dose was 24 g/m². Ara-C was infused alone or in association with anthracyclines or rituximab. The patients were divided in three groups: group A (23 patients with parenteral nutrition without glutamine supplementation), group B (34 patients with parenteral nutrition supplemented with glutamine) and group C (n=15) in which the patients received free diet (sterilized food). The three groups were homogeneous for mean and median Ara-C dose, stem cells support and presence of infectious risk factors (antimicrobial therapy and previous untreated latent infections). In group B, L-Alanil-L-Glutamine supplementation was administered, from January 2001, with parenteral nutrition at the dose of 20 g/day (equivalent to 13 g/day of free-glutamine) for a mean time of 10 days. Bacterial translocations were defined as bloodstream infections with colonic bacteria, where-
as bloodstream infections due to dermal saprophytic bacteria (Staphylococcus spp.) were identified as CVC manipulation related infections. Severe infections were represented by pneumonia, SIRS (severe inflammatory response syndrome) and septic shock. Statistical analysis was performed with $\chi^2$ test with Yates’ correction.

Results. The number of patients who developed infections due to bacterial translocation were 5 in the group A, 6 in the group B and only one in the group C. Staphylococcal bloodstream infections were identified in 11 patients in group A, in 10 patients in group B and in 2 patients in the last group. Five patients in group A, 9 patients in group B and only one in group C developed a severe infection. The total number of patients with infectious complications was 21 in group A (91.4%), 22 in group B (64.7%) and 9 in group C (60%). This difference proved to be statistically significant regarding the difference in the infection rate between group A and respectively B (p<0.025) and C (p<0.05).

Comment. This retrospective study in a cohort of hematologic patients treated with cytarabine based HD-CT showed an association between infectious complications parenteral nutrition without glutamine supplementation. The manipulation of CVC is one of the major cause of bloodstream infection and glutamine may have a role in preventing infections enhancing lymphocyte function and mucosal regeneration. However, due to the low number of patients, this result must be confirmed by prospective randomised trials.

Key words: nutritional support, high-dose cytarabine (HD-Ara-C), infectious complications, CVC.

PO104
HYDRATION WITH SALT REPLETION REDUCES RENAL TOXICITY OF CONVENTIONAL AMPHOTERICIN B EMPIRIC THERAPY: A PROSPECTIVE STUDY ON PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Animal experiments and clinical studies on small number of patients have suggested that hydration and sodium load might reduce amphotericin B-deoxycholate (AmB-d) related nephrotoxicity. We started a study in which a schedule of hydration with electrolyte supplementation was prospectively used in consecutive patients with hematologic malignancies receiving AmB-d treatment to demonstrate whether this strategy might reduce AmB-d related renal toxicity. During the period from December 2000 to August 2002, 77 consecutive patients with hematologic malignancies who received AmB-d therapy, mainly empirically, were prospectively evaluated. Patients received an intravenous hydration of at least 1 L per square meter of body surface, containing at least 1 L of 0.9% saline daily during the whole period of AmB-d treatment. During AmB-d therapy, serum electrolyte concentrations and biochemical parameters of renal function were frequently monitored. When necessary, electrolytes were administered to maintain serum levels within the normal range. Mean serum concentrations of sodium, and phosphorus remained within the normal range during AmB-d therapy. Potassium and magnesium mean serum levels decreased under the normal range only slightly and transiently. The mean intravenous hydration was 2600 mL/day (range 1500-5000 mL/day; mean 1530 mL/day per square meter of body surface). Necessary parenteral potassium and sodium supplementation significantly increased from the first week and from the third week of AmB-d treatment, respectively. Mean creatinine levels remained constant along the whole period of treatment and mean creatinine clearance slightly decreased in the few patients who received more than 3 weeks of therapy. The mean diuresis value was 3350 mL/day (1970 mL per square meter of body surface). Overall, out of 77 patients, 15 patients discontinued AmB-d therapy due to drug-related side effects after 10 mean days (range 1-36 days). Of them, six patients (7.8%) developed renal failure (creatinine levels > 2 mg/dL), one patient developed hepatotoxicity, and in 8 patients AmB-d was discontinued due to infusion related side effects (chills and dyspnea, four cases each). All patients with drug-related side effects recovered after treatment discontinuation. In conclusion, our study represents the first prospective experience in which rules of both hydration and electrolyte supplementation were enclosed in a schedule of AmB-d administration. Our experience seems to confirm that adequate parenteral hydration with a daily urine output of about 2000 mL per square meter of body surface and careful electrolyte supplementation represent simple measures able to contain AmB-d related nephrotoxicity and to permit adequate antifungal therapy at least in the empiric setting.

PO105
THE HEMATOLOGIC HOSPITAL IN THE HOME PROJECT OF THE AZENDA ASL VITERBO (ITALY)

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Important advances are recently achieved in the home care (HC) of patients with hematologic malignancies. As the cost of acute care in hospitals increases, there is a continuous effort to find alternative operating model to assist patients with chronic disease or
those discharged after a planned active treatment who don’t need further intensive in-hospital care. Therefore, the need of reduction of the hospital stay duration and the effectiveness of early discharge schemes, lead the more recent health regulatory laws to stimulate the development of HC services, currently managed most on voluntary basis by the non-profit health care organization (NPHCO). The hematologic continuous HC program of the Hematological Unit of the ASL Viterbo began in 1997, initially supported by a grant of the “Trenta Ore per la Vita” Foundation and then by the Viterbo section of the “Associazione Italiana con le Leucemie ed i Linfomi” (AIL-Viterbo) NPHCO. Over the 1997-2001 years, 215 patients were followed at home. The medical and nursing visits were 545 and 2678 respectively. Most patients were in chronic and advanced phase of disease and presented a wide range of symptoms and complications including fatigue, pain, bleeding and infections. They received intravenous antibiotics therapy, fluids, analgesic treatments and other supportive therapies and symptoms control measures. The patients requiring transfusions were included in the programmed activities of Transfusion Home Team, supported by another NPHCO, as the Viterbo group of the “Associazione Italiana Volontari del Sangue” (A.V.I.S.), that started in 1997 a program of domiciliary blood transfusion for patients affected by chronic diseases and malignancies, who have received until now 3379 red blood cell packets at home. The HC of patients with chronic and advanced hematologic diseases resulted a safe and effective alternative to hospitalisation. Given that we didn’t perform controlled studies, we are not able to report on the improvement of the patient’s quality of life and on the cost-effectiveness of the activity. On other hand, this experience focuses the difficult problems in facing HC organization and the administrative management and the need of integration of the existing services. A close relationship between the in hospital and HC activities has a critical value to avoid the fragmentation of service components and to promote the systematic process to develop case management plans, aimed to improve the efficiency and productivity of interdisciplinary teams and the quality of care. Moving from these results and given the need to unify the two hematologic home services operating behalf the same department, we developed an innovative project, named “Home Hematological Hospital” HHHH, that was submitted to and finally approved by management board of the Local Public Health Agency (ASL Viterbo), according to the national and local health regulatory laws and the planned aims of the Lazio Regional Health Department. The approved projects will allow the admission of the selected patients in HHH, the supply at home of the approved drugs by the nurses and physicians working in the hospital and the reimbursements according to the Italian Diagnosis Related Groups. This approved partnership allows providing the payment of the health operators, based on a fixed fee for each visit, transfusion and other measures, as rehabilitation and psychological assistance, which have not directly managed at home by the ASL Viterbo. To date, the project is in progress and the operative schemes and the decision-making models are just applied. The more definite design of our new organization and the preliminary results will be presented to the meeting.

**PO06 QUALITY OF LIFE AND BRAIN FUNCTION FOLLOWING HIGH DOSE RECOMBINANT HUMAN ERYTHROPOIETIN TREATMENT IN LOW-RISK MYELODYSPLASTIC SYNDROMES**


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**Introduction.** Recombinant human erythropoietin (rHuEPO) has been found to increase hemoglobin (Hb) levels and quality of life (QOL) in patients with cancer-related anemia. rHuEPO has also been shown to improve brain function in patients with end-stage renal disease (ESRD). Primary endpoints of this pilot study were to evaluate the effects of administration of high dose of rHuEPO on QOL and brain function in patients with low-risk MDS and anemia and their relationship with erythroid response. Patients and methods. Eleven patients were included in this prospective, unicentric non-randomized study. Patients (mean age 63, mean Hb level: 8.4 g/dL) had RA (n.4), RARS (n.5) or RAEB (n.2). Three patients had a transfusion-dependent anemia. Patients had to be free of neurological or psychiatric diseases, nor were they treated with neuropsychostatic drugs. rHuEPO (EPREX, Ortho-Biotech) was self-administered subcutaneously at the dosage of 40,000 IU two times a week for 12 weeks. After this period, patients not reaching any erythroid response discontinued therapy. Responsive patients continued with 40,000 IU /week for further 12 weeks. Changes in QOL were assessed by the FACT-An self-report. Neurophysiological evaluation included: duplex scanning of neck vessels, transcranial doppler sonography (TCD) with the study of cerebrovascular reactivity to CO2, neuropsychosudical evaluation, and quantitative Electroencephalography (qEEG). All the patients were studied at baseline (t0), but only 8 completed the neurophysiological evaluation after 24 weeks (t1) (neuropsychological tests and qEEG). Results. Six out of 11 patients (55%) achieved...
an erythroid response at 12 weeks, which was maintained after 24 weeks of treatment. Six patients are still on maintenance therapy after a median follow up of 15 months. No side effects due to rHuEPO were recorded throughout the study. Evaluation of the changes in total FACT-An subscale score showed a relevant improvement from baseline to final evaluation in the majority of responders to rHuEPO, while it did not change in non-responders. The mean 5.14 and 7.8-point increase of FACT-An score observed in responders at 8 and 12 weeks, respectively, exceeded the changes required to reflect a clinical significant improvement in QOL. Seven out of 11 patients had normal values on all neuropsychological tests, whereas two patients had three abnormal tests each, and two patients had two abnormal tests each. In the 8 patients who were re-evaluated at t1, improvement was observed in 3 patients, two of them with abnormal values at t0. No changes were found in 4 patients, two of them with normal values at t0 and two with abnormal values at t0. Worsening was observed in 1 patient, with normal values at t0. Discussion. These data show that a high-dose induction phase with rHuEPO followed by maintenance therapy is an effective and safe therapeutic schedule for low-risk MDS patients, resulting in an erythroid response in more than 50% of treated subjects. Furthermore, the erythroid response was rapid and positively correlated with changes in the QOL. QOL improvement and erythroid response were associated with a positive neurophysiological response in 3 patients, mainly those with altered results at baseline.

**P007**
TREATMENT OF ACUTE RESPIRATORY FAILURE BY NON-INVASIVE VENTILATION IS FEASIBLE AND EFFECTIVE IN HEMATOLOGIC UNITS
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Hematologic patients with acute respiratory failure (ARF), notably hematopoietic stem cell transplantation (HSCT) recipients and patients with concomitant organ failures, have an adverse prognosis, since their mortality rate approaches 100%. Many authors discourage their referral to intensive care unit (ICU) for mechanical ventilation, which is felt as resource wasting. An intermediate strategy is non-invasive respiratory support, with devices delivering positive airway pressure, such as continuous airway pressure (C-PAP), currently employed in various common emergencies, and seems to be promising in hematologic patients. In the last four years, 22 patients affected by various hematologic malignancies (11 male, 11 female; median age 46 years, range 18-69) required C-PAP because of ARF and bilateral pneumonia. Of seven allogeneic HSCT recipients, four were not neutropenic and five were afebrile at the onset of respiratory failure; an infectious agent (Cytomegalovirus) was isolated in only one patient. Of the other fifteen patients (two autologous HSCT recipients), eleven were severely neutropenic and thirteen were febrile at the onset of respiratory distress, with an infectious agent being isolated in nine (Pseudomonas aeruginosa in six cases, Aspergillus fumigatus in two and Cytomegalovirus in one); septic shock was present in four cases. C-PAP was always performed in the hematologic unit. In seven patients (31.8%), including one allogeneic HSCT recipient, respiratory failure resolved with C-PAP only, after a median time of 12 days (range 2-40); six patients were referred to the ICU for mechanical ventilation, with resolution of ARF in one. At day +30, eight patients (36.3%) were alive (two HSCT recipients, one after mechanical ventilation). We distinguish two rather different group of patients. Allogeneic HSCT recipients develop respiratory failure and pulmonary infiltrates when afebrile and after PMN recovery, whereas the other patients are generally febrile and neutropenic, with a causative agent being frequently disclosed. In this latter group we obtained a 40% of success and ventilatory assistance by NIV seems to be mandatory while waiting for the effects of the etiological treatment and PMN recovery. In allogeneic HSCT recipients, the efficacy of ventilatory support is more questionable, but not trivial (28% overall response rate). Many experiences about non-invasive ventilation still imply a strict involvement of the intensive care unit, whereas C-PAP can be easily performed in a hematological department by the local medical and nursing staff, a matter of primary concern in a perspective of cost reduction. Our results are aimed at showing that C-PAP is feasible in an hematologic setting and can help to lead to the resolution of ARF in the presence of bilateral pneumonia, even in severely neutropenic or transplanted patients. The definition of guidelines for selection of patients and timing of C-PAP could address the question regarding the effective rate of responders to this procedure.

**P008**
TRANSDERMAL FENTANYL FOR THE TREATMENT OF PAINFUL ORAL MUCOSITIS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Hematologic patients with acute respiratory failure (ARF), notably hematopoietic stem cell transplantation (HSCT) recipients and patients with concomitant organ failures, have an adverse prognosis, since their mortality rate approaches 100%. Many authors discourage their referral to intensive care unit (ICU) for mechanical ventilation, which is felt as resource wasting. An intermediate strategy is non-invasive respiratory support, with devices delivering positive airway pressure, such as continuous airway pressure (C-PAP), currently employed in various common emergencies, and seems to be promising in hematologic patients. In the last four years, 22 patients affected by various hematologic malignancies (11 male, 11 female; median age 46 years, range 18-69) required C-PAP because of ARF and bilateral pneumonia. Of seven allogeneic HSCT recipients, four were not neutropenic and five were afebrile at the onset of respiratory failure; an infectious agent (Cytomegalovirus) was isolated in only one patient. Of the other fifteen patients (two autologous HSCT recipients), eleven were severely neutropenic and thirteen were febrile at the onset of respiratory distress, with an infectious agent being isolated in nine (Pseudomonas aeruginosa in six cases, Aspergillus fumigatus in two and Cytomegalovirus in one); septic shock was present in four cases. C-PAP was always performed in the hematologic unit. In seven patients (31.8%), including one allogeneic HSCT recipient, respiratory failure resolved with C-PAP only, after a median time of 12 days (range 2-40); six patients were referred to the ICU for mechanical ventilation, with resolution of ARF in one. At day +30, eight patients (36.3%) were alive (two HSCT recipients, one after mechanical ventilation). We distinguish two rather different group of patients. Allogeneic HSCT recipients develop respiratory failure and pulmonary infiltrates when afebrile and after PMN recovery, whereas the other patients are generally febrile and neutropenic, with a causative agent being frequently disclosed. In this latter group we obtained a 40% of success and ventilatory assistance by NIV seems to be mandatory while waiting for the effects of the etiological treatment and PMN recovery. In allogeneic HSCT recipients, the efficacy of ventilatory support is more questionable, but not trivial (28% overall response rate). Many experiences about non-invasive ventilation still imply a strict involvement of the intensive care unit, whereas C-PAP can be easily performed in a hematological department by the local medical and nursing staff, a matter of primary concern in a perspective of cost reduction. Our results are aimed at showing that C-PAP is feasible in an hematologic setting and can help to lead to the resolution of ARF in the presence of bilateral pneumonia, even in severely neutropenic or transplanted patients. The definition of guidelines for selection of patients and timing of C-PAP could address the question regarding the effective rate of responders to this procedure.

**P008**
TRANSDERMAL FENTANYL FOR THE TREATMENT OF PAINFUL ORAL MUCOSITIS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Hematologic patients with acute respiratory failure (ARF), notably hematopoietic stem cell transplantation (HSCT) recipients and patients with concomitant organ failures, have an adverse prognosis, since their mortality rate approaches 100%. Many authors discourage their referral to intensive care unit (ICU) for mechanical ventilation, which is felt as resource wasting. An intermediate strategy is non-invasive respiratory support, with devices delivering positive airway pressure, such as continuous airway pressure (C-PAP), currently employed in various common emergencies, and seems to be promising in hematologic patients. In the last four years, 22 patients affected by various hematologic malignancies (11 male, 11 female; median age 46 years, range 18-69) required C-PAP because of ARF and bilateral pneumonia. Of seven allogeneic HSCT recipients, four were not neutropenic and five were afebrile at the onset of respiratory failure; an infectious agent (Cytomegalovirus) was isolated in only one patient. Of the other fifteen patients (two autologous HSCT recipients), eleven were severely neutropenic and thirteen were febrile at the onset of respiratory distress, with an infectious agent being isolated in nine (Pseudomonas aeruginosa in six cases, Aspergillus fumigatus in two and Cytomegalovirus in one); septic shock was present in four cases. C-PAP was always performed in the hematologic unit. In seven patients (31.8%), including one allogeneic HSCT recipient, respiratory failure resolved with C-PAP only, after a median time of 12 days (range 2-40); six patients were referred to the ICU for mechanical ventilation, with resolution of ARF in one. At day +30, eight patients (36.3%) were alive (two HSCT recipients, one after mechanical ventilation). We distinguish two rather different group of patients. Allogeneic HSCT recipients develop respiratory failure and pulmonary infiltrates when afebrile and after PMN recovery, whereas the other patients are generally febrile and neutropenic, with a causative agent being frequently disclosed. In this latter group we obtained a 40% of success and ventilatory assistance by NIV seems to be mandatory while waiting for the effects of the etiological treatment and PMN recovery. In allogeneic HSCT recipients, the efficacy of ventilatory support is more questionable, but not trivial (28% overall response rate). Many experiences about non-invasive ventilation still imply a strict involvement of the intensive care unit, whereas C-PAP can be easily performed in a hematological department by the local medical and nursing staff, a matter of primary concern in a perspective of cost reduction. Our results are aimed at showing that C-PAP is feasible in an hematologic setting and can help to lead to the resolution of ARF in the presence of bilateral pneumonia, even in severely neutropenic or transplanted patients. The definition of guidelines for selection of patients and timing of C-PAP could address the question regarding the effective rate of responders to this procedure.
Oral mucositis is a major side effect of the high-dose chemotherapy and/or radiotherapy required before hematopoietic stem cell transplantation (HSCT). Most patients experience oral pain, which can be severe, inhibit nutritional intake, cause psychosocial distress and require opiate analgesics. The aim of this study was to assess the efficacy of the transdermal therapeutic system (TTS) of fentanyl administration in treating the pain related to oral mucositis in HSCT patients. Seventy-five adult patients with different hematologic malignancies treated with autologous or allogeneic HSCT were consecutively enrolled in this open trial between November 2000 and December 2002. Thirteen patients not experiencing oral mucositis were excluded from the analysis, which therefore considered a total of 62 HSCT patients (17 allografts, nine non-myeloablative HSCTs, 36 peripheral blood cell autografts). During hospitalisation, all of the patients underwent a weekly oral examination to assess the degree of oral mucositis using the scoring system proposed by the WHO. Furthermore, starting from the transplant day, they were asked to record the intensity of their oral symptoms using a visual analogue scale (VAS). Fentanyl TTS was administered at the patient’s request and renewed every 72 hours if necessary. Twenty patients did not require fentanyl TTS (group A). The first 22 patients asking for the patch received fentanyl 25 µg/hour (group B), and the subsequent 20 patients received 50 µg/hour (group C). The mean pain score (MPS) used to indicate the mean of the VAS scores of all of the patients in the same group for a day seemed to vary in all three groups. There were no significant differences in pain relief between groups B and C. No side effects (nausea, vomiting, sedation, drowsiness, hypoventilation) were observed. In conclusion, we did not find the expected effect of a decrease in the MPS after the application of fentanyl TTS, which therefore did not seem to offer any clinical advantage in the short-term treatment of post-HSCT oral mucositis pain.

PO009
EVALUATION OF THROMBOTIC AND INFECTIOUS COMPLICATIONS OF CENTRAL VENOUS CATHETERS IN PATIENTS WITH HEMATOLOGIC MALIGNANCY: A SINGLE CENTER EXPERIENCE

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Indwelling central venous catheters (CVCs) are essential devices in the management of patients with hematologic diseases being treated with chemotherapy. Thrombosis and infections are the most recurrent complications, particularly in patients with severe and long-lasting neutropenia and thrombocytopenia. Patients and methods. We retrospectively analyzed CVC-related complications in 188 CVCs inserted in 133 hematologic patients who were consecutively admitted to the Hematology Department of Niguarda Ca’ Granda Hospital between May 2002 and April 2003. Hematologic diseases were acute leukemia (91 patients, 48.4%), lymphoproliferative disorders (45 patients, 23.9%), multiple myeloma (35 patients, 18.6%), myeloproliferative syndromes (11 patients, 5.9%), others (6 patients, 3.2%). The CVCs used were polyurethane Plastimed three lumen 7 Fr (92 cases) for chemotherapy and polyurethane Arrow three lumen 12 Fr (94 cases) for chemotherapy and PBSC apheresis. All CVCs were inserted, according to the physician’s judgement, at the patient’s bedside (186 cases) or in the operating room (2 cases) and were used for drug infusion in 162 cases (86.2%), for parenteral nutrition in 12 cases (6.4%), for blood transfusions in 127 cases (67.6%), for peripheral stem-cell apheresis in 47 cases (25.0%) and for through-line blood sampling in all cases. In 19 cases (10.1%) patients received antithrombotic prophylaxis with low molecular weight heparin because of previous thrombosis. Results. The median duration of CVCs after placement was 25 days (range 2-70) for the 7 French type, and 9 (range 1-27) for the 12 French type. No major hemorrhagic complications were related to the insertion procedure. Thrombotic complications developed in 6 patients (3.2% of CVCs). Thrombophilia tests were performed in 4 patients with thrombosis and all tests were negative. Catheter occlusions were observed in 9 cases (4.8%). The frequency of all bacteremias was 20.2% (38 cases) of which 25 were CVC-related (13.2%). Eight cases (4%) had exit and/or tunnel infection. Gram positive bacteria were isolated in 24 cases (63.1%), 15 of which were CVC-related. Gram negative were isolated in 13 cases (34.2%), of which 7 were CVC-related. Candida was isolated in one case. At univariate analysis significant risk factors for infection (p<0.0001) were the number of days/catheters, presence of neutropenia, and high-dose chemotherapy administration.
This study was conducted to evaluate the effects of a new epoetin α dosing regimen on transfusion requirements, hemoglobin (Hb) levels, and quality of life (QOL) in 133 patients with low risk myelodysplastic syndrome (MDS) and Hb <10 g/dL. MDS subtypes were RA 62%, RARS 24% and RAEB 14%. Seventy-nine patients were transfusion-independent having received no transfusion within the previous 3 months, and 54 patients were transfusion-dependent. Epoetin alfa 40,000 IU was given subcutaneously twice weekly; after 4 weeks, the dose could be reduced to 40,000 IU weekly in patients achieving erythroid response. QOL was assessed using the FACT-An questionnaire at baseline and at 4- week intervals. One hundred twenty-nine patients were evaluated for efficacy at week 4 and 126 at week 8, and 124 patients completed the 8-week treatment as planned; responders at week 8 continued the study for up to week 24. The overall response rate at week 8 was 68% (including 48% major responses): 74% in transfusion-independent patients (including 44% major responses), and 59% in transfusion-dependent patients (including 55% major responses). Normal (>12 g/dL) Hb values were obtained in 12 patients (9%) after 4 weeks and in 21 patients (17%) after 8 weeks. In transfusion-independent patients, Hb increased from 8.89 g/dL at baseline by 0.97 g/dL at week 2, 1.53 g/dL at week 4, and 2.08 g/dL at week 8. In transfusion-dependent patients, transfusion requirements were reduced from an average 2.4 RBC units/month before the study to 0.9 RBC units/month over both weeks 1-4 and weeks 5-8. FACT-An scores increased on average by 7.5 after 4 weeks and by 8.8 after 8 weeks compared with baseline. FACT-An scores were positively associated with Hb values. Mean FACT-An score increase at week 8 was 10.2 in responders and 5.6 in non-responders. FACT-An score increases at week 8 calculated in individual patients as percent of baseline value were on average 15% in responders (transfusion-independent 17%, transfusion-dependent 12%) and 6% in non-responders (13% and 1%, respectively). Treatment was generally well tolerated. Our data provide new and encouraging results regarding the benefits of 40,000 IU bi-weekly induction doses followed by 40,000 IU weekly in correcting anemia, reducing transfusion requirements, and improving QOL in low-risk MDS patients.
Persistent neutropenia (p<0.0001) whereas age, diagnosis, status of disease, previous treatment, days in hospital, prophylaxis, type of infection, antibiotic therapy and ESR did not. In the hematologic patients pneumonia is a frequent complication which poorly responds to the first line antibiotic therapy. Therefore, patients often need a second line of antibiotics or addition of antifungal agents with a prolonged hospital stay. Moreover, this complication is associated with a higher mortality rate than that reported for other infections in neutropenic patients because factors affecting outcome, such as neutropenia trend and inflammatory response, do not seem modifiable by the current therapeutic strategies.

PO112
RISK-BASED STRATEGY IN THE MANAGEMENT OF COMMUNITY ACQUIRED FEBRILE NEUTROPENIA: A PROSPECTIVE STUDY
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Hospitalisation for clinical evaluation and intravenous antibiotic therapy is the current standard for treatment of febrile neutropenia. However, in recent years several experiences seem to show the feasibility of an outpatient management in subsets of patients (pts) who are at low risk for the development of infection-related complications. Recently, an internationally validated scoring system to identify low risk patients has been published (Klastersky et al. Clin Oncol 2000; 18: 3038-51). According to this scoring system, pts with a score > 21, calculated on the basis of the presence of specific risk factors, are considered at low risk of complications. Despite a common agreement in the criteria for identification of low risk subjects, the eventual management strategies need to be further investigated. We performed a prospective study on the overall management of neutropenic pts with hematologic malignancies who developed fever while outpatient. The above scoring system was employed for a risk-based management strategy. All consecutive pts with community acquired febrile neutropenia (PMN <500/mm³) admitted at the Hematologic Emergency Unit in the period March 2001 - September 2002 were enrolled in the study. Pts were submitted to standard diagnostic procedures and treated with the antibiotic association ceftriaxone plus amikacin. Pts who responded to the initial antibiotic therapy and were in stable clinical conditions were early discharged. Oral cefixime (400 mg once a day) was used as outpatient continuation antibiotic therapy in the presence of the following criteria: a defervescence lasting more than two days, a score >21, no pulmonary infection, no microbiologically documented infection by a pathogen in vitro resistant to cefixime. Pts who did not fulfill the above criteria completed antibiotic therapy at hospital or were eventually discharged with an intravenous antibiotic therapy. Overall, 100 pts were enrolled in the study. They were 73 males and 27 females; the mean age was 53.5 years (range, 16-78 y); 31 pts were affected by acute leukemia, 48 by non Hodgkin lymphoma and the remaining 21 pts by other malignancies; 70 pts were receiving oral prophylaxis with ciprofloxacin; 58 pts had severe neutropenia (PM N <100/mm³). Febrile episodes were of unknown origin in 71 cases, a bloodstream infection was documented in 13 cases and a pulmonary infection was documented in 16 cases. Six patients died. Out of 94 pts who were discharged after 5.8 mean days of hospitalisation (range 2-30 days), 23 had completed the antibiotic therapy, 53 continued antibiotic therapy with oral cefixime for 4 mean days, and the remaining 18 pts continued intravenous antibiotic therapy for a mean of 4.7 days. Overall, out of 877 cumulative days of antibiotic therapy, in 500 (66%) days the treatment was administered during hospitalisation, and in 300 (34%) days in an outpatient setting after early discharge. Of note, outpatient antibiotic therapy was performed with oral cefixime in 214 (71%) cumulative days. Out of 53 pts who were early discharged and continued antibiotic therapy with oral cefixime, only 2 required hospital readmission due to fever recurrence. In conclusion, our study seems to confirm that hospitalisation and early discharge in the presence of low risk criteria is a feasible clinical approach for patients with community acquired febrile neutropenia. Shift from intravenous to oral (cefixime) antibiotic therapy is a safe and effective practice with advantages in term of the quality of life and cost saving.

PO113
OCCURRENCE OF SEPSIS DUE TO VANCOMYCIN-RESISTANT ENTEROCOCCUS IN PATIENTS WITH ACUTE LEUKEMIA
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In the last decade, the increased prevalence of gram positive sepsis has caused a larger use of glycopeptide antibiotics for microbiologically documented infections as well as for empiric therapy. The emergence of glycopeptide-resistant gram+ strains represents a relevant problem in hematologic clinical practice. Nosocomial infections from vancomycin-resistant enterococcus (VRE) are of special interest. It has been recently report-
ed that pre-transplant VRE colonization is associated with bacteremia at high death risk. It is meaningful that similar episodes can occur in patients with hematologic neoplasia, long hospitalization and severe cytopenia. On this background, we evaluated the prevalence of enterococcus sepsis in patients with acute leukemia followed in our institution in the last 2 years. From January 2001 to November 2002, we observed 9 episodes of enterococcus infections out of 49 sepsis cases (16%). Namely, in 2001 five cases were from E. Faecalis with 2 VRE and in 2002 three from E. Faecium and one from E. Faecalis, with one VRE. Both strains of E. Faecalis were isolated in patients with relapsed acute myeloid leukemia, previously heavily treated and with severe and prolonged neutropenia. Empiric antibiotic therapy with ceftazidime, amikacin and teicoplanin was ineffective and both cases died for complications related to cytopenia and sepsis. In the case with E. Faecium infection the empiric antibiotic combination was ineffective, while the replacement of ceftazidime with piperacillin-tazobactam resulted in prompt resolution of fever and sepsis. Microbiologic cultures demonstrated that E. Faecium strain was teicoplanin, vancomycin, β-lactamase-resistant and oxazolidinone-sensitive. Strain were studied by PCR with specific primers for faecium and faecalis types and for genes of VAN A and VAN B resistance. The results of these assays demonstrated that all strains had VAN A gene and that phenotype study with E-test was always concordant with genotypic data. Molecular studies showed that E. Faecalis strains were not clonal. In addition, in the general patient population hospitalized in our unit we found a prevalence of 20% carriers of enterococcus spp, with 6% of them VRE, as assessed by rectal swabs. The present survey confirms that VRE infections in neutropenic patients represent an emerging problem because of the possible antibiotic resistance. Thus, the microbiological surveillance is of increasing relevance in order to identify cases at greater risk for enterococcus infection.

P0114
INFECTIOUS COMPLICATIONS FOLLOWING NONMYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Non-myeloablative hematopoietic stem cell transplantation (NST) has been explored in hematologic malignancies and solid tumors in an attempt to minimize treatment-related toxicity. Whether this approach is associated with reduced risk of infectious complications is unclear. The aim of the current study was to evaluate infectious complications in a series of 39 consecutive adult patients who received NST at our institution. Peripheral blood stem cell grafts (n=37) or marrow grafts (n=2) were infused from HLA-matched sibling (n=37) partially matched related (n=1) or unrelated (n=1) donors. Seventy-four percent of the patients underwent NST following fludarabine-based reduced conditioning protocols. Neutropenia developed in two thirds of patients and lasted 16 days. Acute GVHD grade II to IV was observed in 25% of patients, whereas 35% of patients have signs of extensive chronic GVHD. Twenty-four patients (61%) had at least one significant infectious episode. Bacteriaemia occurred in 15% of patients (n=5 Gram-positive, n=1 Gram-negative microorganisms). CMV infection was observed in 14 out of 26 (39%) evaluable patients; 4 of these had recurrent or persistent CMV antigenemia requiring a second-line treatment, but eventually cleared the viremia. No patients experienced CMV disease. Fungal infections were documented in 6 (15%) patients, comprising invasive fungal infections in 3 cases (pulmonary aspergillosis) and mucosal fungal infections in three (Torulopsis glabrata). Six patients died of transplant-related causes, and 4 of these died before day +100. Infection was considered the primary cause of death in 1 patient (pulmonary aspergillosis) and contributed to death in another two. The actuarial probability of non-relapse mortality at 100 days was 10% (95% CI, 3%-26%). Our preliminary results suggest that NST is associated with a low incidence of bacterial, fungal and viral infections. Whether these findings would translate into an improved overall survival need to be confirmed in larger prospective studies.

P0115
TOXOPLASMA GONDII INFECTION IN THE MANAGEMENT OF HEMATOLOGICAL MALIGNANCIES. A GIMEMA-INFECTION REPORT
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GIMEMA, Infection Program

Objectives: To evaluate the real impact of Toxoplasma gondii infection in the management of patients with hematological malignancies, or undergoing transplant procedures. Design: A retrospective study, conducted over a ten-years period (1993-2002). Setting: Twenty-one Hematology Divisions in tertiary care or university hospitals participating to GIMEMA-Infection program. Results: Toxoplasmosis was diagnosed in 6 patients. The reported patients suffered for Hodgkin’s disease (2 cases), Non-Hodgkin lymphoma (1 case), acute lymphoblastic leukemia (2 cases), and acute myeloid leukemia (1 case); all of them but one had undergone a previous transplant procedure (2 autoPBSCT, 3 alloBMT).
In 1 case Cryptococcus neoformans infection and toxoplasmosis was diagnosed contemporaneously. Only 4 patients presented at the occurrence of infection a low absolute neutrophil count lower than 0.5x10^9/L. Signs of infection were fever in 5 cases, in 4 patients together with neurological signs such as seizure, cephalalgia, and projectile vomiting; 1 patient presented neurological signs only, and in the last case the infection caused fever only (in this patient the diagnosis was achieved only at autopsy). All the patients underwent a radiological examination, by CT scan or MRI, that resulted suggestive for toxoplasmosis in 5 cases. A proper diagnosis was achieved in only 3 patients, by polymerase-chain reaction (PCR) on cerebral tissue, cultural examination of liquor, and autopsy; in the other cases, since the negativity of serological examinations, the determination of the infectious agent was presumptive, on the basis of the symptomatology and of the radiological findings. Three patients received an empirical wide-spectrum antimicrobial treatment at the onset of fever; in all but one patient a specific treatment with pyrimethamine and sulfadiazine or spiramycin was administered when the etiological diagnosis was made; 1 patient did not receive any antimicrobial therapy. Four patients achieved an improvement, with complete or partial reduction of the lesions; 1 patient achieved an initial but not lasting response; the last patient died. At day 30 from the diagnosis of infection, all patients were alive; after a more prolonged follow-up, only in 1 case, Toxoplasma accounts for death, after 4 months since diagnosis. Conclusion: Toxoplasmosis is a rare complication in HIV negative patients; its serological detection and diagnosis remain difficult since the IgM response may be lacking, and diagnosis can be presumptively based on neurological signs and radiological findings (mostly by MRI), which are usually characteristic. In conclusion, on the basis of our retrospective survey, we retain that toxoplasmosis is a rare event in the management of hematological malignancies, with a low clinical relevance.

One of problems in communicating the diagnosis to patients is the frequent discrepancy on the way to communicate among the different professionals involved in the care of patients. This produces not clear messages to the patient who frequently do not trust to the medical staff or get in a depression and relatives look for other centre. In order to show the dimension of this problem we planned a study, actually ongoing, finalized to disclose were the discrepancies emerge and in order to minimize this aspect we studied the possibility to standardise the way of communicating and to recognise critical moments in which the way of communicating has to be changed. The method includes an interview done to 100 adult patients as inpatient’s clinic of which 50 already discharged and 50 during the hospital stay; in order to avoid conditioning the interviews are planned with a medical doctor and psychologist not involved in the initial communication of diagnosis. The interview was performed at the patient. The interview is divided into 3 different phases: 1) the level of knowledge of the diagnosis and is significance before the admission in the hospital, 2) the level of understanding after the communication in the hospital, 3) the status of the patient after the communication. A second step is performed in those patients who demonstrate discrepancies in the answers and it is finalised to show if the patients are in the condition of understanding, would like to understand or could understand and how these substantial differences are perceived by the staff members. The preliminary data available in 20 patients shows that almost 50% of patients have a non correct perception of diagnosis; it seems to be that this altered perception of the communication involves either the modality of the communication per se due to non adaptation of the language to the culture of the patient either the interest of patient to know. Due to few data now available this interesting aspect has to be further investigated. We believe to be able to present all data in the contest of the Congress.

Vertebral fractures are the most important source of morbidity in patients with multiple myeloma (MM). Nonoperative treatments such as cytotoxic drugs, radiotherapy, analgesic medications or bisphosphonates are increasingly used but none is uniformly effective in relieving pain. Surgical management generally involves vertebrectomy and reconstruction with PMMA (poly-methylmetacrylate) bone cement, but it is not indicated...
for the treatment of patients with multifocal vertebral lesions. Another treatment for vertebral fractures is vertebroplasty, which involves the percutaneous injection of PMMA into a fractured vertebral body; however this procedure does not reexpand a collapsed vertebra, it can just reinforce and stabilize fracture and it is rarely related to leakage of PMMA through cortical defects, with epidural compression of neural elements. A recent modification of vertebroplasty is the percutaneous balloon kyphoplasty (PKB). In March 2003 we performed a percutaneous kyphoplasty in a 73 years old male affected by MM (IgA k), with multiple fractures in L4 and L5 vertebral bodies (VB). He underwent to a chemotherapy accorded to scheme VAD and he achieved a good partial remission. The persistent use of analgesic medications (transdermal fentanyl) and bisphosphonates were ineffective in relieving pain. Kyphoplasty was performed on L5 VB (that was quite collapsed) after induction of general anesthesia. A 13-gauge needle was introduced trough a small dermatotomy and advanced to the posterior aspect of each pedicle along its superolateral cortex. The needle was directed anteriorly, medially and caudally through the pedicle. Using a hand-mounted drill, the bilateral channels were created to reach the posterior one third of VB. Through the channel a high-pressure balloon was introduced and inflated to reduce the VB back to its original height; so we created a cavity that was subsequently filled with the PMMA. Balloon inflation and the PMMA filling were performed under fluoroscopy vision. After a month the patient was well and stopped analgesic medications. In conclusion, in this case kyphoplasty was effective in relieving pain and improving the patient’s quality of life. More cases are necessary to evaluate the safety and efficacy of this procedure.

PO118
INTERMITTENT NON-INVASIVE VENTILATION IN IMMUNOCOMPROMISED PATIENTS WITH ACUTE RESPIRATORY FAILURE

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Avoiding intubation is a major goal in the management of acute respiratory failure in immunocompromised patients, affected by hematologic malignancies. Transfer to the intensive care unit, endotracheal intubation and mechanical ventilation are associated with a significant risk of death. The early use of intermittent non-invasive ventilation (NIV) during acute respiratory failure can help to avoid the need for endotracheal intubation and improve the outcomes of patients. We conducted a feasibility study of intermittent NIV, in 10 hematologic pts at an early stage of hypoxicemic acute respiratory failure: in these patients immunosuppression was caused by neutropenia after intensive chemotherapy (grade IV, 4/10 pts), bone marrow transplantation (2/10 autologous, 2/10 allogeneic) or it was a result of intensive corticosteroid therapy (2/10 pts). The enrolled patients showed: pulmonary infiltrates; fever; severe dyspnea at rest; a respiratory rate of more than 30 breaths per minute; a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen (PaO2:FiO2) of less than 200 while the patient was breathing oxygen through a Venturi mask. Non-invasive ventilation was delivered to the patients through a helmet. The helmet was adjusted and connected to a Venturi meter with a pressure support of oxygen. Positive end-expiratory pressure (PEEP) was repeatedly increased by 2 cm of water (up to a level 10 cm of water) through a valve. The end point of each procedure was to maintain the arterial oxygen saturation above 90 percent. The period of non-invasive ventilation lasted at least 60 minutes and alternated every 2-5-10 hours, according the arterial oxygen saturation. The mean duration of NIV was 8 days (range 6-11). During the first 4 days, the NIV was administrated for a mean of 5-6-9 and 6 hours respectively; subsequently the mean duration of NIV was 5 hours per days. Pts stopped NIV when the PaO2:FiO2 ratio was more than 200 while the patient was breathing oxygen through a Venturi mask. Eight patients overcome the respiratory failure: 4 of them were discharged; two patients died for cardiac failure and two for progressive disease. Two patients died for respiratory failure in fourth and seventh day respectively after the beginning of NIV. We think that the beneficial effects of PEEP on alveolar recruitment and in treating atelectasia at an early stage of respiratory infection help to maintain adequate alveolar ventilation during the acute respiratory failure. In our study intermittent use of NIV was possible without transferring the patients to the intensive care unit and allowed endotracheal intubation to be avoided, with encouraging results.

PO119
PSEUDOMONAS AERUGINOSA INFECTIONS: EPIDEMIOLOGY AND FACTORS AFFECTING OUTCOME IN A SINGLE TERTIARY CARE CENTER

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Several centers have described infections due to multiresistant Pseudomonas aeruginosa (PA) defined as
resistant to all antipseudomonal agents such as quinolones, penicillins, cephalosporins and carbapenems. We described 46 infections by PA occurred during a 8-years survey in order to study epidemiology, factors affecting resistance acquisition, response to antibiotic therapy and outcome. Twenty-five (54%) were male, the median age was 58 years (range 15-78) and 33 (72%) had acute leukemia. Thirty-five patients received chemotherapy while 11 underwent stem cells transplantation. All patients were hospitalised in HEPA filter rooms, 35 (76%) received prophylaxis with quinolones and granulocyte growth factors were given to 33 patients (72%). The median neutrophils count at fever onset was 75/microliter (range 0-13000/µL) and the median duration of prior neutropenia was 5 days (range 0-26). Isolates were multiresistant, according to above definitions, in 16 cases (35%); 13/16 multiresistant PA were isolated during summer season. Overall, ciprofloxacin, ceftriaxim, imipenem and piperacillin/tazobactam sensitivities were 26%, 37%, 35% and 50%, respectively. Multivariate analysis showed that the only factor related to multiresistant PA isolation was the days of previous exposure to quinolones (> 8 days; p = 0.021). Bloodstream infections occurred in 22 cases (48%), 2 (4%) patients had pneumonia. 3 (6.5%) had soft tissue infection and 19 infections (41.5%) involved more than one of the above mentioned sites. Five patients (11%) had septic shock syndrome and four (9%) developed disseminated intravascular coagulation. First line therapy was successful in 13 cases (28%) without significant differences as regard monotherapy, combination therapy or use of different classes of antibiotics. Second line therapy was successful in 18 patients (39%). Overall, the median duration of fever, antibiotic therapy and hospital stay were 6 days (range 2-35), 18 days (range 6-35) and 26 days (range 7-45), respectively. Factors negatively affecting response to first line therapy were > 2 days from fever onset to PA laboratory isolation (p = 0.010) and type of infection other than isolated bacteremia (p = 0.008). Sixteen patients (35%) died from PA infection and the probability of surviving at 30 days was 65%. Factors associated with poor outcome resulted lack of neutrophils increase (p = 0.0001) and lack of response to first line antibiotic therapy (p < 0.0001) whereas multiresistant PA isolation had no impacts (p = 0.351). The probability of surviving at 30 days was 100% when either a neutrophils increase or a response to first line antibiotic therapy was achieved while they were 48% (p = 0.0011) and 51% (p = 0.0037), respectively, when they were not. In summary, the emergence of multiresistant PA may be prevented limiting the prophylaxis with quinolones. Infection by PA involving many sites remains a life-threatening complication independently of sensitivity of PA to antibiotics; prompt specific therapy and strong supportive care were essential to improve outcome. However, since we are not able to modify prognostic factors such as infection localization and neutrophil recovery so far, we should make every effort to prevent the nosocomial spread of multiresistant PA in the environment using appropriate hygienic measures and having trained hospital personnel.

ANAEROBIC BACTEREMIA IN PATIENTS WITH LEUKEMIA AND LYMPHOMA. SEVERE OROPHARYNGEAL MUCOSITIS AND STATUS OF HEMATOLOGIC DISEASE (RELAPSED OR REFRACTORY) ARE THE MOST IMPORTANT PREDISPOSING CONDITIONS

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Anaerobes are one of the most important components of the normal human skin and oropharyngeal flora and can be a cause of bacterial infections of endogenous origin in immunocompromised hosts. An increasing number of anaerobic infections in this setting has been recently reported and often the type of anaerobes isolated suggests an oropharyngeal source of these infections. Herein we report our experience about anaerobic bacteremia (AN). Over a 10-year period (from January 1992 to December 2001) 34 episodes of AB were identified in 34 different patients (16 males, 18 females, median age 35, range 17-69) for a rate of 0,5 AB per 100 patient admissions; it accounted for 4% of all bacteremic episodes that occurred in our Department during the study period. The majority of pts had a refractory/relapsed leukemia (20/26) or refractory/relapsed lymphoma (5/8); 5/34 (15%) received a BMT procedure. 28/34 (82%) pts were neutropenic at onset of AB with a WBC count less than 100 cells/mmc and 26/34 (76%) had severe oral mucositis following intensive chemotherapy (grade III°-IV° WHO in 20/26 cases). Bacteremic episodes occurred after a median of 16 days of severe neutropenia (range 5-28) and of these 8/34 (24%) were polymicrobial. Pulmonary infiltrates were observed in 4/34 (12%) pts. The Fusobacterium nucleatum was the most frequently isolated pathogen (in 18 cases), followed by Bacteroides species (in 8 cases), Peptostreptococcus species (in 5 cases) and Clostridium species (in 3 cases). The most common antibiotic treatment was piperacillin-tazobactam (13 pts), carbapenems (10 pts) and glycopeptide plus cefazidime (4 pts). The median duration of antibiotic therapy was 9 days (range 3-18). The response rate of first line antibiotic therapy was 72% and the mortality AB related was 5/34 (15%). To identify risk factors for infections a retrospective case-control study was performed (for each case 2 control pts). Univariate analysis revealed that the severe mucositis (WHO III°-IV°) and the status of refractory/relapsed disease were significantly associated to development of AB. No signif-
icant differences were found with regard to chemotherapy regimen, duration of neutropenia, empirical antibi-otic treatment, antibacterial prophylaxis, invasive procedures. Conclusions. 1) In our experience Fusobac-terium sp., Bacteroides sp., Peptostreptococcus sp., and Clostridium sp., are the most common isolates causing anaerobic bacteremia in onco-hematologic patients. 2) Conditions predisposing to AB include severe oropharyngeal mucositis and refractory/relapsed status of oncohematologic disease; in these cases the use of antibiotic therapy with activity against anaerobes should be considered, especially in those patients do not respond to initial empirical antimicrobial treatment.

**PO121**

**ACUTE B19 HUMAN PARVOVIRUS INFECTION, COMPlicated BY PURE RED CELL APLASIA AND THROMBOTIC THROMBOCYTOPenic PURPURA, TERMINATING WITH A FULMINATING MYOCARDITIS: A CASE REPORT**

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Human Parvovirus (HPV) B19, the causative agent of erythema infectiosum, can cause a wide spectrum of disease syndromes, may be associated with anemia, aplastic crisis, thrombocytopenia, granulocytopenia, acute and chronic arthritis, myocarditis and hepatitis. The cellular receptor for HPV B19 has been identified as blood group P antigen and it serves to explain the tropism of this virus for replicating erythroid cells. This antigen has also been found on endothelial and fetal myocardial cells. Both acute and persistent courses of B19-infections have been reported. Infections of the heart, relatively common and asymptomatic, usually evolve into a spontaneous and complete resolution. It can, however, in rare cases, lead to substantial cardiac damage and congestive heart failure. We report on the case of a young patient with an acute B19 HPV infection who presented with pure red cell aplasia (PRCA), thrombotic thrombocytopenic purpura (TTP), hemophagocytic syndrome (HPS) and a rapidly fulminating myocarditis. Case report: a 34-year-old woman was admitted because of fever and severe malaise and skin rashes. The physical examination revealed no other abnormalities. The lungs were clear, the abdomen was soft, the liver and the spleen were not palpable; the neurologic examination was not remarkable. The X-ray of the chest and the cardiac ultrasonographic study were normal. A complete blood count revealed normocytic anemia, low reticulocytocemia and thrombocytosis. The apoglibin level was increased (441 U/L); lactate-dehydrogenase (LDH), bilirubin and complete coagulation assessment resulted in normal values. The Coomb's tests were negative. Specimens of blood, urine and stool, obtained at different times, were negative. An intravenous broad-spectrum antibiotic therapy was begun without any benefit. The patient progressively deteriorated and additional steroid therapy was given without any improvement of her clinical status. On the fifteenth day after the admission, the patient presented an exacerbation of malaise, acute painful polyarthopathy, altered mental status and renal dysfunction. The laboratory tests showed increased LDH (6450 mU/mL), severe thrombocytopenia (platelet count 8,000/mL) and the exacerbation of the anemia. The peripheral blood smears numerous schistocytes were found. These findings were consistent with a diagnosis of TTP. The examination of the bone marrow aspirate revealed a marked erythroid hypoplasia with the disappearance of more mature erythrocyte precursors and no other abnormalities, suggesting a PRCA, confirmed by the serologic evidence (Ig M specific antibodies) of B19 HPV infection. Treatment with fresh frozen plasma by continuous infusion was promptly started. Because of sudden deterioration of her general condition, the patient was performed plasma-exchange. She presented a rapidly progressive heart failure and the typical laboratory features of a disseminated intravascular coagulation syndrome. A cardiac ultrasonographic re-evaluation showed several signs of severe myocardial damage. Circulating serum cardiac autoantibodies were negative. A diagnosis of myocarditis was suspected and the patient, being unable to perform an endomyocardial biopsy, died two days later. An autopsy was performed confirming the dilative myocardopathy and evidencing disseminated thrombotic occlusions of the microcirculation, above all in the kidneys and in the brain, and the typical pathological features of a HPS. The HPV B19 was detected into myocardial cells by molecular (RT-PCR) and immunohistological analyses. This report, confirming the very extended tropism of this virus, which caused a complex syndrome in our patients with polymorphic clinical manifestations and a fatal course, suggests that clinicians should be alerted to the possible role of HPV B19 in myocarditis presenting in immunocompetent patients.
Pre-emptive therapy (ganciclovir and foscarnet) based on detection of CMV infection in the blood before disease develops, is an effective strategy developed to reduce the risk for CMV-associated sickness and death. However, after primary pre-emptive therapy ends, about 30%-50% of patients (pts) can be expected to have CMV relapse. Cidofovir (CDV) is a nucleoside analogue with broad in vitro activity against CMV and has the practical advantage of weekly administration. However, there are limited data on the secondary pre-emptive use (HSCT pts who failed or relapsed after primary pre-emptive therapy) of CDV in CMV infections. In view of this, we performed a study to evaluate the efficacy of CDV as secondary pre-emptive therapy for CMV infections in HSCT recipients who failed or relapsed after pre-emptive therapy. Patients and methods: we treated with CDV 9 allogeneic HSCT recipients with a median age of 20 years (range 6-47), affected by AML (5 pts), ALL (2 pts), MM (1 pt), and lymphoblastic lymphoma (1 pt). The graft type was: bone marrow (6 pts) and cord blood cells (3 pts). The donor type was: HLA-identical sibling donor (4 pts), and unrelated donor (5 pts). All but one recipients were CMV seropositive before HSCT. The 9 patients who failed (5 pts) or relapsed (4 pts) after previous pre-emptive therapy (ganciclovir or foscarnet) received intravenous CDV using a dosage of 5 mg/kg once a week for the first 2 weeks followed by 2 doses every other week, for a total of 4 doses. All patients received probenecid and prehydration according to manufacturer’s recommendations. Patients were weekly monitored for CMV DNA by quantitative DNA hybridisation test (Murex Hybrid Capture CMV DNA assay (HCA)) and by quantitative pp65 antigenemia. Response to CDV was defined as normalisation of pp65 antigenemia and of HCA test remaining normal for at least 3 consecutive weeks after discontinuation of CDV therapy. Failure to CDV was defined as the persistence of CMV DNA and/or of pp65 antigenemia at the time of discontinuation of CDV therapy. Results: 7 of 9 (77%) pts responded to CDV revealing a complete clearance of CMV; before starting CDV therapy, the median pp65 antigenemia level was 3 pos. cells (range 1-8 pos. cells) and the median CMV DNA viral load was 700 copies/mL (range 700-9332 copies/mL). The remaining 2 pts failed to respond to CDV therapy; before starting CDV therapy, both pp65 antigenemia (1 and 2 pos. cells, per patient, respectively) and HCA test (700 and 1170 CMV DNA copies/mL, per patient, respectively) were slightly positive. Both pts were again treated with ganciclovir or foscarnet. Overall, the 9 pts were well tolerated CDV therapy, and renal toxicity was not observed. Conclusions: our data encourage the use of cidofovir in larger studies to prove the efficacy of this antiviral agent in allogeneic HSCT recipients who failed or relapsed after pre-emptive therapy.

P0123

MOLECULAR EPIDEMIOLOGY OF PSEUDOMONAS AERUGINOSA WITH AMPLIFIED FRAGMENT LENGTH POLYMORPHISM IN STEM CELL TRANSPLANTATION PATIENTS

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Pseudomonas aeruginosa is still responsible for significant morbidity and mortality rates in severe immunocompromised patients and is a major cause of nosocomial infections. Several putative risk factors have been identified, as transmission from patients, from the hospital environment to patients, from hospital personnel via hands, contaminated infusions and from sanitary facilities. From October 2001 to June 2002, we documented 6 cases of Ps. aeruginosa sepsis in stem cell transplantation patients: 1 ABMT, 2 HLA identical sibling donors, 2 matched unrelated donors, 1 partially-matched (4/6) cord blood. All patients were allocated in single positive-pressure rooms with HEPA filtered air and private bathroom until discharge. All strains, isolated from patients which were allocated in the same rooms in different periods, had identical antimicrobial susceptibility pattern. Consequently, environmental specimens were collected from hospital furniture, surfaces, sanitary facilities and water and phenotypic analysis were performed. Antimicrobial pattern of specimens, obtained from bathrooms and from blood of patients, were similar. To verify the hypothesis of the single source of infection, 15 isolates (from patients and from bathrooms) and the ATCC 27853 Ps aeruginosa strain (internal control), were typed employing AFLP (chromosomal DNA, amplified fragment length polymorphism). The amplification products were resolved by electrophoresis on an automatic sequencing apparatus. Computer-aided cluster analysis of the AFLP results with Gel Compar 3.0 software showed that among the 15 isolates, 3 different genetic groups with similarity > 84% were present: A) 4 environmental isolates, B) 1 isolate from a patient, C) 5 isolates from patients and 5 from environment. These data suggest that molecular similarity between the strains isolated from patients and from the environment (bathrooms) led us to emphasize the importance of environmental contamination as a common source of infection and possible nosocomial transmission. Although the epidemiology of Ps. aeruginosa remains complex and not fully elucidated, genomic analysis is an important method for epidemiologic investigation and development of rationale prevention measures.
PO124
CRYPTOCOCCOSIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES. EXPERIENCE OF GIMEMA-INFECTION
GIMEMA Infection Program

Objective: To evaluate the clinical, laboratory characteristics and outcome of patients with cryptococcosis complicating hematological diseases. Design: A retrospective study, conducted over a ten-years period (1993-2002). Setting: Twenty-one Haematology Divisions in tertiary care or university hospitals. Results: The study included 17 patients (m/f 12/5; median age 61 years) with hematologic diseases (6 AML, 3 NHL, 2 ALL, 2 MDS, 1 each for CLL, HD, MM, and CMML) who developed a cryptococcosis. Among these, only 4 patients underwent stem cell transplantation (2 BMT, 1 aBMT, 1 PBSCT). Analyzing possible risk factors we recognized that before the onset of infection: 6 patients received steroids for the underlying malignancy; 4 patients suffered for diabetes mellitus; 2 had cutaneous wounds and 1 each had respectively an autoimmune disease, an hepatic cirrhosis, a chronic renal failure and exposure to pigeons. Five patients received an oral prophylaxis, in only 2 cases with fluconazole. Symptomatology was characterized by fever, neurological and respiratory signs reflecting the primary sites of infection (5 blood, 4 CNS and lung respectively, and 1 each gut, skin and mouth). Diagnosis was made with antigen detection in serum or CSF, positive microbiological culture or PCR. All patients started a specific treatment (7 patients with fluconazole and 10 with deoxycolate or liposomal amphotericin B). Only 2 patients died from cryptococcosis within 30 days from diagnosis. Conclusions: Cryptococcosis represents a very rare complication in patients with hematological malignancies. It is characterized by a low lethality if compared to that observed in the other fungal infections in neutropenic patients (i.e. aspergillosis or mucormycosis). A specific treatment, timely started, is able to positively influence the outcome.

PO125
QUALITY OF LIFE IN A GERM-FREE ENVIRONMENT: DISTRESS AND PSYCHOPHYSICAL PAIN PERCEPTION
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A germ-free single room is an isolated space-time environment. In this special unit, bone marrow transplantation (BMT) treated patients receive intensive and individualized care which contributes to their feeling of security. Environmental stressors can influence emotional stability and perceived autonomy. The purpose of this study was to analyze the effect of isolation on the quality of life, distress and psychophysical pain perception in BMT treated patients. The Experiential World Inventory (EWI) of Bonneau and El Meligi was distributed to 13 consecutive patients (7M, 5F, mean age 40 years) at the beginning and end of isolation (from 32 to 60 days, mean 45). Patients received bone marrow from related or unrelated donors, after reduced or standard conditioning regimens. The Questionnaire contains 400 items and investigates 12 distinct scales: sensorial perception, time perception, body image, self-concept, social behaviour, thought, dysphoria, impulsiveness, hyper and hypoesthesia, euphoria, anxiety. A control group of 20 normal subjects confirmed the reference values reported in the Italian edition of the EWI. The differences registered at the beginning and end of isolation were significant (p<0.05) for the perception of time, body image, social behaviour, impulsiveness and euphoria scales. Our results show that prolonged isolation in a germ-free single room may induce progressive impairment of sensorial perception and body image, acceleration of time perception with reduced coping abilities, negative evaluation of self and a tendency to communicate less with others. A better understanding of predisposition to distress and psychophysical pain perception, careful monitoring of psychological stability and reduction of environmental stressors, may improve coping in BMT-treated patients.

PO126
HEMATOLOGIC HOME CARE:SUPPORTIVE THERAPY AND CHEMOTHERAPY IN PATIENTS AFFECTED BY ACUTE LEUKEMIA
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From 1992 Hematological Home Care (HHC) was organized as an institutional activity supported by Associazione Italiana Leucemie. One of the purposes of this activity was the improvement of quality of life for patients with refractory/resistant disease or ending in death patients. From June 1992 to May 2003, 250 patients entered the HHC service: 119 patients (48%) were affected by acute leukemia (103 patients presented AML and 16 presented ALL). Twenty-two patients were ending in death; 13 patients discharged from hospital and needed to continue home supportive therapy such as transfusions and antibiotics. These patients have to be readmitted to the hospital in the future for further treatments (two of these patients were treated by HD-ARAC at home, three patients were...
Eighty patients presented refractory/resistant leukemia: 41 patients were treated only by supportive therapy; 70% of these patients presented PS 3-4, the median age was 66 years (range 16-92), the median assistance period was 72 days (range 3-425). During HHC 78 patients were treated by palliative chemotherapy: 40 patients were treated by 98 cycles of monochemotherapy IV or SC, 38 patients were treated by oral chemotherapy; 84% of patients presented PS 2-3, the median age was 63 years (range 16-86) and the median assistance period was 187 days (range 6-925). The supportive therapy was characterized by 1144 transfusions of RBC, 177 platelet aphereses and 75 cycles of intravenous antibiotics. All patients during the period of HHC have presented an infective or hemorrhagic complication which could have induced admission to hospital; 85% of these events were resolved at home. During HHC 91 patients died, 69 patients (86%) died at home.

HEMOSTASIS, THROMBOSIS, PLATELET DISEASES

PO127
HEMATOLOGIC HOME CARE PROGRAM: AN INNOVATIVE PROJECT TO IMPROVE THE QUALITY OF LIFE IN HEMATOLOGIC PATIENTS
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Every year the Haematology Division of Niguarda Hospital in Milan takes care of about 800 new patients suffering from oncological and hematologic pathologies. For a global approach and tailored care we organised a home care program. A project started in 2001 and became fully effective in 2002 after a pilot period of about one year. Design and Methods. Two kinds of patients were considered eligible for the program: a) chronic patients who were generally hospitalised or attended Day Hospital; b) acute patients who were discharged after chemotherapy treatment or after infection complications. Patients requiring blood tests, i.v. therapy administration, infusion pump regulation for continuous infusion treatment, parenteral nutrition, catheter use management and medication were included; periodic clinical controls were carried out by nurses and hematologists. Results. From 1/01/02 to 30/04/03 41 patients, 22 F and 19 M, aged between 21 and 90 years (median 68 years), were assisted. Among them 2 had hemolytic anemia, 3 acute leukemia, 9 lymphoproliferative syndromes, 15 multiple myeloma, 12 myelodysplastic/myeloproliferative syndromes. The median duration of home care assistance was 46 days (range 1-245) for a total of 1901 days. Two hundred and eighty one home visits were provided (107 in 2002 and 173 in 2003 first quarter) for the following: 112 blood tests, 75 i.v. therapy infusions (antibiotic, diphosphonate, steroids and other drugs), 34 central venous catheter managements, 38 nurses visits, 58 medical examinations, 4 blood transfusions. Interpretation and Conclusions. The characteristics of patients suffering from malignant diseases and the lack of significant hematologic home care experience caused several difficulties at the beginning of the project; however the progressive increase in the number of patients and their positive opinions about this approach encouraged the operators to continue this project, despite the problems related to the management of these severely ill patients. Haematological home care turned out to be a feasible kind of assistance which enables us to improve the quality of life and to reduce average costs of care for each patient.
Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disorder characterized by intravascular hemolysis and life-threatening venous thrombosis. Prophylactic anticoagulation treatment in all patients could improve survival and reduce morbidity. Patients and methods. Thirteen patients (males = 2, females = 11; mean age 46 years, range 26-65) with well documented PNH underwent prospective evaluation of antithrombin, protein C, free and total protein S, activated protein C resistance. In all patients the presence of antiphospholipid antibodies was investigated by kaolin clotting time (KCT), diluted Russel's viper venom time (DRVVT) and by research of anticardiolipin antibodies IgG and IgM (ACA-G and ACA-M). Prevalence of factor V Leiden, prothrombin variant G20210A and thermolabile variant C677T of methylenetetrahydrofolate-reductase was evaluated. The same parameters were evaluated in 100 normal subjects (Males = 50, Females = 50; Mean age 45 years, range 24-69) who constituted the control group and in 10 patients (males=5, females=5) with diagnosis of aplastic anemia.

Results. Five patients (38.4%) had history of thrombotic events vs no case in control group (Fisher's test = p < 0.0001) Antithrombin, protein C and protein S were normal in all cases. No patients had factor V Leiden, prothrombin variant G20210A and thermobalable variant C677T of methylenetetrahydrofolate-reductase was evaluated. The same parameters were evaluated in 100 normal subjects (Males = 50, Females = 50; Mean age 45 years, range 24-69) who constituted the control group and in ten patients (males = 2, females = 11; mean age 46 years, range 26-65) with well documented PNH underwent prospective evaluation of antithrombin, protein C, free and total protein S, activated protein C resistance. In all patients the presence of antiphospholipid antibodies was investigated by kaolin clotting time (KCT), dilute Russel's viper venom time (DRVVT) and by research of anticardiolipin antibodies IgG and IgM (ACA-G and ACA-M). Prevalence of factor V Leiden, prothrombin variant G20210A and thermolabile variant C677T of methylenetetrahydrofolate-reductase was evaluated. The same parameters were evaluated in 100 normal subjects (Males = 50, Females = 50; Mean age 45 years, range 24-69) who constituted the control group and in 10 patients (males=5, females=5) with diagnosis of aplastic anemia.

Results. FV-L was observed in 1 patient (1.4%), FIIA20210 mutation in 2 patients (2.8%) and MTHFR C677T variant in 12 (16.6%) patients. The prevalence of these mutations was not statistically different from that observed in the general population from the same ethnic background. Twenty-six (36.1%) patients had hyperhomocysteinemia (>95% above reference values), partially related to the presence of TT MTHFR homozygosity. As far as natural anticoagulants are concerned, 6 patients (9.5%) showed low levels of protein S, while no patient showed deficit of protein C or AT. Low titer of aCL was found in 2 (3.1%) cases. Six patients (8.3%) (2 ALL, 2 AM L and 2 AM L-M) developed TE in the first month of follow up. Two out of six patients (33.3%) showed the association of FV-L or FIIA20210 mutations and hyperhomocysteinemia. The OR for TE in patients with double defects was 11.0 (95% CI 1.4-85.8, Fisher exact test, p = 0.048). Adults patients with AL and presence of double prothrombotic defects appear to be at high risk for the development of TE.
Thrombotic Risk among Patients with Philadelphia-Negative Chronic Myeloproliferative Disorders Carriers of Thrombophilic Gene Polymorphisms

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Philadelphia-negative chronic myeloproliferative disorders (CMDs) (polycythemia vera, PV, and essential thrombocythemia, ET) are associated with a well-established thrombotic risk. In order to evaluate whether the carriership of common gene polymorphisms associated with thrombophilia play a role in the development of thromboembolic disease we studied 181 patients with CMDs (M/F 90/91, median age 52 years, range 17 to 92). Diagnosis was PV in 84 cases and ET in 97 cases. All patients were genotyped for the presence of factor V Leiden and prothrombin G20210A. Membrane glycoprotein (GP) Ia/IIa mediates platelet adhesion to collagen and the C807T polymorphism in the GP Ia gene is correlated with a variable expression of the platelet surface receptor, being the TT genotype associated with a higher receptor density; 111 patients of the present series were genotyped also for the presence of such polymorphism. Sixty-two patients (34.2%) suffered from a vascular occlusive event, involving the arterial system in 40 cases (22.1%) and the venous system in 22 cases (12.1%). Factor V Leiden and/or prothrombin G20210A were present in 11 patients (17.7%) with arterial (7) or venous thrombosis (4) and in 6 asymptomatic patients (5%, relative risk 5.2, 95% CI 1.7 to 15.6). The relative risks for arterial (3.5, 95% CI 1.2 to 9.7) or venous events (3.6, 95% CI 1.1 to 11.7) among carriers were similar. The distribution of the TT, CT, and CC genotypes in the GP Ia gene was similar among patients with thrombotic disease and among asymptomatic individuals. In conclusion the 807 TT polymorphism in the GP Ia gene does not increase the thrombotic risk in patients with CMDs. The magnitude of the risk for venous thromboembolism (VTE) associated with factor V Leiden or prothrombin G20210A is similar to that found among patients with VTE and no CMDs in comparison with normal subjects. The presence of factor V Leiden or prothrombin G20210A appears to be associated in this setting with a moderate increase in risk for arterial occlusive events, unlike the patients with arterial thromboembolism and no CMDs.
Background. The P2X1 receptor is a fast ATP-gated cation channel that is present in blood platelets. Its role in platelet activation has been difficult to study because it undergoes rapid desensitization by ATP released during the preparation of platelet-rich plasma (PRP). Recent studies have shown that the non-hydrolysable P2X1 agonist, αβ-methylene-ATP, induces a transient platelet shape change in platelet preparations to which the ATP degrading enzyme apyrase was added to prevent receptor desensitization. Further studies performed using the traditional light-transmission aggregometer have indicated that P2X1 may have a marginal role in platelet aggregation induced by threshold concentrations of collagen. However, these studies may not reflect accurately the situation at sites of vascular injury, where platelets interact with thrombogenic subendothelial components under flow conditions that generate variable levels of shear stress, which are highest in small arterioles and, in pathological conditions, at the top of atherosclerotic plaques partially occluding the arterial lumen. Aim of the study was to investigate the role of the platelet P2X1 receptor in shear-induced platelet aggregation. Methods. Using a cone-and-plate viscometer that allows monitoring platelet aggregation in real-time, we studied the role of P2X1 in shear-induced platelet aggregation (SIPA) using PPACK-anticoagulated human PRP containing apyrase (1.5 U/mL), which prevented P2X1 desensitization. The results were compared to those obtained 30 sec after the addition of alpha-beta-methylene-ATP (10 μmol/L) to apyrase-PRP, which caused P2X1 desensitization. Results. Platelet aggregation in apyrase-PRP started after a lag-phase of 15.5±7.7 s (n=8) from the application of high shear stress (108 dynes/cm2) and the signal measuring light transmission reached a maximal amplitude of 3.8±2.5 nW. In contrast, after P2X1 desensitization with alpha-beta-methylene-ATP, platelet aggregation was delayed (lag phase, 36.3±15.2, p<0.001) and its amplitude was decreased (1.9±1.5, p<0.01). The mean concentrations of platelet factor 4 (PF4) 30 s after the application of shear stress were 388±92 ng/mL in platelet-poor plasma (PPP) from apyrase-PRP and 274±69 in PPP from apyrase-PRP after P2X1 desensitization with alpha-beta-methylene-ATP (p=0.02), indicating that P2X1 plays a role in platelet secretion induced by high shear stress. Conclusions. It is well established that shear-induced platelet aggregation is initiated by the interaction of soluble von Willebrand factor (VWF) multimers with glycoprotein and there is evidence that it is amplified by the interaction of released ADP with its P2 receptors. These results now indicate that P2X1 stimulation by released ATP may play an important role in accelerating and amplifying VWF-mediated platelet secretion and aggregation under flow conditions that are relevant for normal hemostasis and, particularly, pathological thrombosis.

PO133 Efficacy and Safety of Splenectomy in Autoimmune Thrombocytopenic Purpura: Long-Term Results of 402 Cases

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Chronic autoimmune thrombocytopenic purpura (ATP) is an autoimmune disease characterized by increased platelet clearance caused by anti-platelet autoantibodies, which bind circulating platelets, resulting in destruction by the reticuloendothelial system. Corticosteroids (CS) is the therapy of first choice with complete (CR) or partial (PR) response in about 70-80% of cases. However, ATP patients begin refractory to CS or other immunosuppressive drugs in the majority of cases. Even though splenectomy (spl) is effective in about 70-80% of these patients, few data are available concerning its long-term results. We retrospectively analyzed from the archives of 22 Italian hematological centres the available data of 402 patients; 137 males and 265 females, with a median age at diagnosis of 34 yrs (range 1-84) who underwent to laparotomic splenectomy for ATP. Median follow-up after splenectomy was 57 months (range 1-498); 121 patients have a follow-up>120 months. 387 patients were splenectomized after 1 or more therapeutical approaches and 15 patients had no prior treatment (4 at diagnosis and 11 during follow-up). The median number of platelet at splenectomy was 25×10^9/L (range 1-406), with a median interval of 14 months (range 1-384) from diagnosis to splenectomy. 163 (40%) patients received polyvalent pneumococcal vaccine. Complete response (CR) was defined when platelet (PLT) >150×10^9/L, partial response (PR) when PLT 50-149×10^9/L and no response (NR) when PLT<50×10^9/L or an increase less than 30×10^9/L respect...
to the baseline value after 1 month from splenectomy. 345 pts (66%) showed a good response (CR+PR) after splenectomy. 79 of these (23%) relapsed with a median time from splenectomy of 8 months (range 2-236). Most of the relapses (80%) were seen within 48 months after splenectomy. 68/79 were subsequently treated with a variety of other agents and a good response was observed in 46 (68%) cases. 57 (16%) patients were refractory and 53 of these were subsequently treated with a variety of other agents and a good response was observed in 23 cases. Three patients (1 relapsed and 2 refractories) died for intracranial hemorrhage after 149, 105 and 10 months from surgery. 12 patients (2%) experienced a thrombotic complication after splenectomy, fatal in three cases (all pts had a normal platelet count at that time). 33 patients (8.2%) experienced a major non fatal infectious complication during the follow-up (16 pts close to splenectomy); the incidence of infection among patients that received polyvalent pneumococcal vaccine was 6.7% (11/163) and 9.2% (22/239) among not vaccinated patients. We evaluated various pre and postsplenectomy variables for their usefulness in predicting a favorable sustained response or risk of relapse after splenectomy. In a multivariate model using logistic regression the platelet number after seven days from surgery and the number of various treatments presplenectomy were predictive of favorable response and only the platelet number after seven days from surgery was predictive of relapse. This report including one of the largest cohort of patients described so far, confirms the efficacy and safety of splenectomy in ITP. The long-term follow-up allow us to demonstrate the low risk of relapse after four years from splenectomy, confirming the potential of splenectomy to provide long-term control of disease.

P0134

IDIOPATHIC THROMBOCYTOPENIC PURPURA IN THE ELDERLY:
CLINICAL COURSE IN 179 PATIENTS

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Idiopathic thrombocytopenic purpura (ITP) is often diagnosed in the elderly (age / 65 years). We retrospectively analyzed 178 patients (males: 81, females: 97, mean age 72 years, range 65-87) affected by ITP diagnosed from 1981 to 1998. At diagnosis, mean platelet count was 55×10^9/L (range 1-139×10^9/L). Bleeding symptoms were present in 73/178 (41%). Bleeding symptoms were classified according to a score system based on four severity categories. In detail, 105 patients did not show bleeding symptoms; 52 had score 1; 19, score 2; 2, score 3, no patients had score 4. At diagnosis 112/178 (63%) (males: 60, females: 52, m/f 1.17, mean age 71 years, range 65-85, mean platelet count: 79.5×10^9/L, range 23-139×10^9/L) were initially observed without any therapy; 91 patients did not present bleeding symptoms; 16 had score 1; 5, score 2. In 66/178 (37%) patients, (males: 21, females: 45, m/f 0.46, mean age 72 years; range 61-87) therapy was started at diagnosis. At the beginning of therapy mean platelet count was 15×10^9/L (range 1-43×10^9/L). Fourteen patients did not show bleeding symptoms; 36 had score 1; 14, score 2; 2, score 3. Ninety/112 (80%) remained under observation during a long follow-up (median 36 months). Among these, 5/90 patients (5.5%) showed a spontaneous remission, without therapy. Seventy-three/90 (81.1%), maintained platelet count between 50 and 150×10^9/L; 12/90 (13.4%) maintained platelet count below 50×10^9/L without occurrence of bleeding symptoms and without therapy need. Of the 112 patients under observation, after a median follow-up of 20 months, only in 11/112 (9.8%) therapy was started either for decrease of platelet count from baseline levels or for appearance of bleeding symptoms. At the beginning of therapy, mean platelet count was 18.5×10^9/L. range 11-23×10^9/L; 2 patients showed bleeding score 0; 1, score 1; 6, score 2; 2, score 3. Eleven/112 (9.8%) patients, after a median follow-up of 47 months, showed the occurrence of other pathologies. Four antiphospholipid antibodies (APA) syndrome, 3 myelodysplastic syndrome (MDS), 1 MALT gastric lymphoma, 1 rheumatoid arthritis, 1 monoclonal gammopathy of uncertain significance (MGUS), 1 ovarian carcinoma. A total of 77 patients have been treated (66 just after diagnosis and 11 after a period of follow-up). Prednisone was the first-line treatment in all these patients (mean daily dose 0.43mg/kg, range 0.1-1.5). One month after therapy start in 49/77(63.6%), was obtained complete (platelet count >150×10^9/L) and partial response (platelet count >50×10^9/L); in 28 (36.4%) no response (platelet count <50×10^9/L). Thirty-one/49 (63.3%) initially responding patients, maintained their first response after a median follow-up of 31.3 months. Fourteen/49 (28.6%) responding patients relapsed after a median time of 13 months. Four/49 responding patients showed the occurrence of other pathologies after a median period of follow-up of 46.85 months: two MDS, 1 chronic lymphocytic leukemia (CLL) and 1 bladder carcinoma. One no responder patient showed the occurrence of a gastric carcinoma (median follow-up 59.3). Three/14 relapsed patients showed the occurrence of other pathologies after a median period of follow-up of 116.5 months: 1 MGUS, 1 pancreatic carcinoma, 1 APA syndrome. In all cases the first-line therapy was prednisone, given at mean daily doses much lower than standard doses used in younger adults. It is noteworthy that initial overall response rate was above 60% and that responses (40%) tended to be persistent. Prednisone seems to be an
appropriate initial treatment also in elderly patients. In fact, at the last control, 31 of 49 (63.3%) initially responding patients, maintained their first response. At the same time an adequate follow-up of elderly patients with reduced platelet count is very important, because of the possibility that isolated thrombocytopenia could not be a real ITP, defined as idiopathic immunologic platelet hyperdestruction, but, on the contrary, a disease due to production defect, or secondary to another pathology.

The mean TPO plasma level of Hypoplasia group was significantly higher than the other groups (F=29.75; p<0.001) of patients with thrombocytopenia. Plotting a ROC curve for the diagnosis of thrombocytopenia secondary to bone marrow decrease of megakaryocytes, the sensitivity and specificity of TPO assay was 73% and 90% respectively with a threshold level of 170 pg/mL. The TPO median levels of patients with PV and ET was not statistically different from normal. Conclusions: assay of plasma TPO seems to be a useful tool in the diagnosis of thrombocytopenia since a level above 170 pg/mL is suggestive of a megakaryocytes mass reduction.

The transcription factor GATA-1, together with its cofactor FOG-1, regulates erythropoiesis and megakaryopoiesis. Mutations occurring in the FOG-1 face of GATA-1 are responsible for dyserythropoietic anemia with thrombocytopenia, while R216Q, the only mutation identified in its DNA face, induces X-linked thrombocytopenia with thalassemia (XLTT). The former disorder has been investigated in detail, while little is known about XLTT since only one family has been investigated. We recently studied a second family (the propositus, a male, and two females) with R216Q mutation. The propositus had splenomegaly and petechiae with a prolonged bleeding time and an imbalanced globin chain synthesis. Routine analyses showed a mild anemia and high reticulocyte count that, together with low aptoglobin and elevated LDH plasma levels, were indicative of peripheral hemolysis. Molecular studies on globin loci excluded the presence of defects in the β-globin gene or rearrangement within the α-globin cluster. Hematopoietic colony assay and morphological examination of the propositus’ bone marrow showed mild dyserythropoietic aspects. Both differentiation and proliferation of megakaryocyte (Mks) were unaffected in vivo and in vitro, but maturing elements were severely dysmorphic and few of them reached the stage of terminally differentiated and platelet-elaborating Mks. Immunofluorescence revealed that actin was abnormally distributed in Mks, but normally organized in the erythroid line. Optical and electron microscopy showed that several platelets were round and

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Posters : Infections, Quality of Life, Support Therapy

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large and characterized by the absence or paucity of granules, abundant and dilated surface connected canalicular system, as well as an abnormal distribution of tubulin. Surface glycoproteins and in vitro aggregation of platelets were normal, whereas shape change and clot retraction were defective. Imbalanced globin chain synthesis was the only relevant defect in females. Our study confirms the role of R216Q in XLTT. Affected males present a mild dyserythropoiesis resulting in an imbalanced globin chain synthesis and peripheral hemolysis, associated to a severe defect of Msks maturation inducing quantitative and qualitative platelet abnormalities. Obligate female carriers are not easily distinguishable from subjects with mild forms of β-thalassemia, thus raising a new problem of differential diagnosis.

PO137
THE TREATMENT OF THE THROMBOTIC THROMBOCYTOPENIC PURPURA BY ROSE’S SCORE
Casula P, Di Tucci A, Usala E, Mamusa A, Angelucci E
Hematology Division and BM T Unit, “A. Businco” Hospital, Cagliari, Italy

Introduction: We report in this paper our experience about the diagnosis and the treatment of the TTP. The patients were treated according to the Rose’s score. The patients were treated according to the Rose’s score at diagnosis. Rose’s Score was similar that reported in the literature. Deaths were correlated to Rose’s score at diagnosis. Rose’s Score was not related to relapse risk. Relapse risk persists for at least 10 years after first event.

PO138
RISK FOR RECURRENT VENOUS THROMBOEMBOLISM AFTER A FIRST SUPERFICIAL VEIN THROMBOSIS AMONG CARRIERS OF INHERITED THROMBOPHILIA
De Stefano V, Rossi E, D’Orazio A, Za T, Leone G
Institute of Hematology, Catholic University, Rome, Italy

The association of inherited thrombophilia with superficial vein thrombosis (SVT) has been previously demonstrated in a collaborative investigation (Thromb Haemost 82,1215,1999). However, no information is available about the long-term management of thrombophilic patients having suffered from SVT of the legs as heralding event. We investigated 205 patients with SVT (M/F 67/138, median age at the thrombotic event 36 years, range 15 to 74 years), in comparison with a control group of 703 healthy individuals (M/F 410/293, median age 41 years, range 7 to 93). A preliminary evaluation had ruled out patients with overt neoplastic or autoimmune disease. Diagnosis was established by ultrasonography in all cases, ruling out the coexistence of a deep vein thrombosis (DVT). In 91 patients (44%) SVT was associated with a circumstantial risk factor (pregnancy in 13 cases, puerperium in 30, oral contraceptive intake in 11, surgery in 12, trauma in 17, bed rest in 4, other causes in 4). All individuals were genotyped for the presence of factor V Leiden (FV-GA) and the G20210A polymorphism in the prothrombin gene (PT-GA). Moreover all the patients underwent a diagnostic panel including measurement of antithrombin (AT), protein C (PC), and protein S (PS). Among the controls 21 individuals carried FV-GA (3%, 20 heterozygotes and 1 homozygote) and 21 were heterozygous for PT-GA (3%). Among the patients 73 (36%) had inherited thrombophilia (1 AT deficiency, 8 PC deficiency, 10 PS deficiency, 37 FV-GA, 11 PT-GA, 6 combined defects), with an associated risk for SVT 9.0 times higher than in the controls (95% CI 6.0-13.8). One hundred and fifty-three patients had an observation time between the first SVT and the referral to our Thrombosis Center or a recurrent event longer than 1 year and were considered eligible to retrospectively evaluate the risk for recurrent venous thromboembolism (VTE); the observation time of the patients with thrombophilia (n= 63, 41%) and those without (n= 90, 59%) was similar (median 5 and 6 years, respectively). No significant increase in the risk for recurrent VTE was found among carriers of inherit-
ADP-induced \([\text{Ca}^{2+}]_i\) increase in fura-2AM-loaded, haematologica. Increase in free cytoplasmic calcium (\([\text{Ca}^{2+}]_i\)) is necessary for normal ADP-induced platelet aggregation. P2Y1-driven Gq and the P2Y12-driven Gi pathways is necessary for normal ADP-induced \([\text{Ca}^{2+}]_i\) increase. M. Maggiore, University of Milan, Italy

Department of Internal Medicine, IRCCS Ospedale Paolo, Department of Medicine, Surgery and Dentistry; °Unit of Hematology and Thrombosis, Ospedale San Cattaneo M, °Lombardi R, °Lecchi A*

*Unit of Hematology and Thrombosis, Ospedale San Paolo, Department of Medicine, Surgery and Dentistry; °ABianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Ospedale Maggiore, University of Milan, Italy

**PO139**

**CONCOMITANT ACTIVATION OF BOTH THE P2Y1 DRIVEN Gq AND THE P2Y12 DRIVEN Gi PATHWAYS IS NECESSARY FOR NORMAL ADP-INDUCED MOBILIZATION OF PLATELET CYTOPLASMIC CALCIUM**

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*Unit of Hematology and Thrombosis, Ospedale San Paolo, Department of Medicine, Surgery and Dentistry; °ABianchi Bonomi Hemophilia and Thrombosis Center, Department of Internal Medicine, IRCCS Ospedale Maggiore, University of Milan, Italy

Background. Concomitant activation of both the P2Y1-driven Gq and the P2Y12-driven Gi pathways is necessary for normal ADP-induced platelet aggregation. It is generally accepted that the ADP-induced increase in free cytoplasmic calcium ([Ca\(^{2+}\)]_i) is mediated by the P2Y1 receptor only. However, the first reported patient (VR) with P2Y12 deficiency (Cattaneo et al., Blood 1992;80:2787) had borderline-low increases in platelet [Ca\(^{2+}\)]_i induced by ADP, suggesting that P2Y12 might play a role in ADP-induced intracellular Ca\(^{2+}\) mobilization. Aim of the study was to evaluate whether P2Y12 plays a role in the ADP-induced intracellular Ca\(^{2+}\) mobilization. Methods. We studied the ADP-induced [Ca\(^{2+}\)]_i increase in fura-2AM-loaded, washed platelets from 17 normal subjects and 2 patients with congenital P2Y12 deficiency (VR and MG). The experiments were performed in the presence of EDTA (1 mmol/L) and in the presence or absence of the antagonists for P2Y1 (AP25P 0.5 mmol/L) or P2Y12 (AR-C69931MX 1 µmol/L). Results. The mean (±SD) increase in [Ca\(^{2+}\)]_i induced by ADP (10 µmol/L) in normal platelets was 376±95 nmol/L; it was completely abolished by the P2Y1 antagonist AP25P, while it was only partially inhibited by the P2Y12 antagonist AR-C69931MX (278±6, p<0.01). The ADP-induced increase in [Ca\(^{2+}\)]_i in VR’s and MG’s platelets was borderline-low (130 and 282) and was not further decreased by AR-C69931MX (158 and 266), but was completely abolished by AP25P. The PI-3 kinase inhibitor wortmannin (0.1 µmol/L) did not significantly affect the ADP-induced increase in [Ca\(^{2+}\)]_i in normal or patients’ platelets. In addition, the adenyl cyclase inhibitor SQ22536 (300 µmol/L) did not normalize the ADP-induced increase in [Ca\(^{2+}\)]_i in normal platelets in which the P2Y12-receptor had been blocked by AR-C69931MX. Conclusions. Concomitant activation of both the P2Y1-driven Gq and the P2Y12-driven Gi pathways is necessary for normal ADP-induced [Ca\(^{2+}\)]_i increase. Like for platelet aggregation, the P2Y1-driven Gq pathway triggers the platelet [Ca\(^{2+}\)]_i response, while the P2Y12-driven Gi pathway amplifies it. The mechanism by which P2Y12 amplifies the ADP-induced [Ca\(^{2+}\)]_i increase is still under investigation.

**PO140**

**THROMBOTIC RISK AMONG PATIENTS WITH ACUTE LEUKEMIA**

De Stefano V, Sorà F, Rossi E, Laurenti L, Fianchi L, Pagano L, Sica S, Leone G

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The association of thromboembolic disease and acute leukemia at diagnosis has been anecdotically reported during the last 30 years. Patients with acute lymphoid leukemia are known to have a not negligible thrombotic risk (4% to 10%) related with administration of asparaginase. In order to evaluate the overall thrombotic risk associated with acute leukemia at diagnosis and during treatment we carried out a prospective study. The primary end-point was symptomatic venous or arterial thrombosis diagnosed by objective methods; superficial thrombophlebitis of the arms (possibly due to diagnostic or therapeutic phlebotomy) were not computed as events of interest. We studied 379 patients (M/F 200/179) with acute leukemia enrolled from 1993 to 2002: 310 with acute non lymphoid leukemia (ANLL), 31 of them with M3 leukemia, and 69 with acute lymphoid leukemia (ALL). The median age was 60 years (range 14 to 89). During the observation time 24 thrombotic events were recorded: the median age of the affected patients was 51 years (range 17 to 84). Twelve events (8 deep vein thromboses, DVTs, of the legs, 1 superficial thrombophlebitis of the ST of the leg, and 3 ischemic strokes) occurred among patients with ANLL not-M3 (4.3%), 7 events (1 DVT of the arm, 4 DVTs of the legs, ST of the leg, and 1 cerebral venous thrombosis) occurred among patients with ALL (10.1%), and 5 events (4 DVTs of the legs, and 1 ischemic stroke) occurred among M3 patients (16.1%). The cumulative probability of thrombosis according to the Kaplan-Meier curve was 3.4% at diagnosis, 4.3% after one month, and 7.6% after six months. The Kaplan-Meier
DISEASE AND THROMBOPHILIA
PO141
PULMONARY HYPERTENSION IN PATIENT WITH TYPE I GAUCHER'S DISEASE AND THROMBOPHILIA
Giuffrida G, Figuera A, Cingari R, Burgio N, Di Francesco E, Giustolisi R
Cattedra e Divisione di Ematologia con Trapianto, Ospedale Ferrarotto, Catania, Italy

Gaucher's disease (G.D.) type I (β-glucocerebrosidase deficiency) is characterized by the storage of uncleaved β-glucocerebroside in the cells of the reticuloendothelial system leading to bone marrow infiltration, hepatosplenomegaly and skeletal lesions. Haematological changes with anemia, thrombocytopenia and thrombocytopatie are common. Recently clotting factor and natural inhibitors deficiencies have also been reported but the pathophysiology of such abnormalities is still unclear. We describe a 39-year old male patient with type I Gaucher's disease (GD), diagnosed at 5 years after splenectomy and treated with enzyme replacement therapy (ETR) at the low dosage-high frequency regimen (15 U/kg per month), presenting pulmonary hypertension (PHT) and thrombophilic risk factors. At admission the patient complained rest dyspnea and inferior limbs oedemas. Echocardiography showed a mild right heart dilatation, moderate tricuspid regurgitation and pulmonary hypertension with pulmonary artery systolic pressure (PAPs) at 42 mmHg. Pulmonary-function testing outlined a moderate decrease of single breath diffusing capacity for carbon monoxide (DLC0 = 31.9 mL/mmHg/min corresponding to 65% of the predictive value), and a mild reduction of forced expiratory flows. Arterial-blood gases evaluation indicated a mild hypoxemia with hyperventilatory hypocapnia. Thrombophilic risk factors were evaluated and Factor V Leiden mutation (homozygous) and low levels of protein S (24%-n.v:70-140%) were detected. ETR with Imiglucerase (Cerezime-Genzyme CO) at high doses (60 U/kg per month) and oral anticoagulant therapy with Warfarin was immediately started. Pulmonary involvement was thought to be prevalent only in the more severe cases of the neuronopathic forms (types II and III); however, it was demonstrated an incidence of more than 50% abnormal pulmonary function tests among patients with the non-neuronopathic form (type I). Several possible pathophysiological mechanisms account for lung involvement in GD. Gaucher's cells (GC) can fill the alveolar spaces and/or the inter- and intralobular septa, loading to air space and/or interstitial disease, respectively. The commonest pattern of pulmonary involvement was the presence of Gaucher's cells in the septal capillaries. This phenomenon probably represents release of GC from the bone marrow into the systemic circulation and it has been suggested that the hypertension is due to plugging of alveolar septal capillaries. Another possible mechanism, recently hypothesized, is pulmonary thromboembolism. In our patient thrombophilic risk factors may predispose to pulmonary thromboembolism and to PHT. Few cases of GD type I with PHT are described in literature. In these patients hypercoagulability might act as a precipitating factor for PHT and therefore an useful combination of high dose of ETR and anticoagulant therapy may be indicated.

PO142
ACQUIRED HEMOPHILIA ASSOCIATED WITH MYELODYSPLASTIC SYNDROME
Giuffrida G, Figuera A, Cingari R, Burgio N, Di Francesco E, Giustolisi R
Cattedra e Divisione di Ematologia con Trapianto, Ospedale Ferrarotto, Catania, Italy

Acquired hemophilia (AH) is a rare bleeding disorder caused by an autoimmune depletion of factor VIII C (F VIII:C), due to specific inhibitor. The inhibitor may occur in association with pregnancy or post-partum, autoimmune diseases, medication, solid tumors and hematologic malignancies. An association with lymphoproliferative disorders has been reported, while association with myelodysplastic syndrome (MDS) is extremely rare and only one case, to our knowledge, has been described. Treatment has of two objectives: permanent inhibitor suppression and management of the acute bleeding episode, but no general consensus exists on the best therapeutic approach. Recently investigations suggest that oral cyclophosphamide and prednisone, without FVIII therapy, may be useful in patients with high titer inhibitor. We report a case of AH asso-
associated with MDS treated only with oral immunosuppressive therapy. A 73-year old woman, with a 10-years history of MDS-Refractory Anemia, developed spontaneous soft tissue hemorrhages, hematuria and progressive anemia (Hb 7.5 gr/dL), although platelets number was normal (Plt 207×10⁹). Her family history was negative for hemorrhagic diathesis. Coagulation assay showed a normal prothrombin time and fibrinogen levels and a prolonged activated partial thromboplastin time (APTT 115''-n.v. 34''). FVIII C level was < 1% (n.v. 60-150); lupus anticoagulant’s research was negative. An antibody direct against FVIII C was found at high titre (130 BU/mL). A diagnosis of AH was made and oral immunosuppressive therapy with prednisone 1 mg/kg/day and cyclophosphamide 100mg/day was started. APTT, level of FVIII and inhibitor was measured every 1-week APTT gradually returned to normal value, inhibitor level decreased whereas FVIII levels increased and returned to normal value after 4 weeks (Table 1). One month later, hemorrhagic diathesis disappeared and Hb increased (11 g/dL) without blood transfusions. Cyclophosphamide was stopped after 4 weeks and prednisone was gradually tapered off after 3 months. In patients with MDS has been hypothesized a dysregulation of the immune system that may favour the development of an abnormal lymphoid clone and in our case, probably, autoantibodies against FVIII. In conclusion our observation illustrates high titre inhibitor-AH associated with MDS- Refractory Anemia successfully treated with only oral prednisone and low dose of cyclophosphamide. Causal relationship between MDS and AH remains speculative. Although the clinical course is not predictable and inhibitor may disappear spontaneously, in some cases, with high titre inhibitor associated with malignant disease, combined therapy with prednisone and cyclophosphamide may be sufficient to suppress inhibitor and to arrest bleeding.

Table 1. Coagulation profiles.

<table>
<thead>
<tr>
<th>n.v.</th>
<th>At visit</th>
<th>1st wk</th>
<th>2nd wk</th>
<th>3rd wk</th>
<th>4th wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT (sec)</td>
<td>34''</td>
<td>115''</td>
<td>82</td>
<td>63</td>
<td>45</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>60-119%</td>
<td>&lt; 1</td>
<td>4</td>
<td>41</td>
<td>53</td>
</tr>
<tr>
<td>FVIII inhibitor (BU/mL)</td>
<td>&lt; 0.01%</td>
<td>130</td>
<td>45</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Interference of antiphospholipid antibodies with the anticoagulant activity of the protein C pathway has been suggested to play a role in the development of the thrombotic complications of antiphospholipid syndrome. IgG anti-β2-glycoprotein I antibodies have been shown to inhibit the lipid-dependent inactivation of factor Va (FVa) by activated protein C in plasma. Here, we studied the effect of antiprothrombin antibodies on the inactivation of FVa in a system with purified coagulation factors. Total IgG was isolated from plasma of five antiphospholipid-positive patients and two controls. In all cases, patients’ IgG recognized human prothrombin bound to anionic lipid surfaces as detected by ELISA and ellipsometry, displayed lupus anticoagulant activity in normal and β2-glycoprotein I-depleted human plasma, and inhibited the lipid-dependent human prothrombin activation by the prothrombinase complex in a system with purified coagulation factors. To assess the interference of these antiprothrombin antibodies on the lipid-dependent inactivation of FVa by activated protein C, 1 µM human prothrombin was preincubated with test IgG in the presence of 1 µM lipid vesicles composed of 20 mol% phosphatidylserine and 80 mol% phosphatidylcholine and 3 mM CaCl₂. Subsequently, 0.15 µM protein S, 40 pM Factor Va, and 100 pM activated protein C were added and the residual amount of FVa remaining after 10 minutes was determined. Under these experimental conditions, residual FVa amounted 17-20% when 1.5 mg/mL normal IgG was present. In the presence of the same concentration of patient IgG, the residual FVa ranged from 31 to 82%. The inhibitory effect was strictly dependent on the presence of human prothrombin, ruling out an effect of antibodies - if any - directed against protein S or activated protein C. Furthermore, the inhibitory effect was proportional to the concentration of IgG and was strongly diminished at a 10-20 fold higher lipid concentration. In conclusion, antiprothrombin IgG inhibit the lipid-dependent inactivation of FVa by the protein C pathway in a system with purified proteins. This effect may contribute to the increased risk of (venous) thrombosis in the antiphospholipid syndrome.
PO144
MANAGING CLINICALLY SUSPECTED DEEP VENOUS THROMBOSIS (DVT). THE EXPERIENCE OF ANCONA UMBERTO I-TORRETTE HOSPITAL: THE TVP TEAM
Rupoli S,1 Pulini S,1 Agostini V,1 Argalia G,1 Barulli S,1 Candela M,1 Catalini R,1 Cola G,1 Da Lio L,1 Fraticelli P,1 Paci E,1 Pagliaruccio G,2 Ravaglia F,2 Salvi A,2 Scortechini AR,1 Tassetti A,1 Zingaretti O,1 Leoni P1
1Clinica di Ematologia, 2Radiologia Clinica, 3Radiologia e Diagnostica per Immagini, 4Clinica Medica, 5Clinica di Medicina Interna, 6Clinica di Chirurgia Vascolare Università Politecnica delle Marche, Azienda Ospedale Umberto I, Ancona 7Laboratorio Analisi, 8Medicina Interna, 9Clinica di Chirurgia Vascolare, 10Clinica di Medicina dell’Urgenza Azienda Ospedale Umberto I, Ancona, Italy

Venous thromboembolism (VTE) is the third most common cardiovascular disease; its prevalence exceeds 1 per 1000. To answer the growing demand of integration between clinical, laboratory and instrumental approach to the patient with clinical suspect of DVT in our hospital it has been constituted a thrombosis care unit called TVP-TEAM. The main purposes have been to give patients a preferential way of access to the hospital, an overall and optimized diagnostic process, a timely beginning of the most appropriate therapy and a following continuous management of the oral anticoagulant therapy according to INR, performed by the Anticoagulation Specialized Center. We combined clinical assessment (the standardized clinical probability), D-dimer testing (latex-enhanced turbidimetric assay) and colourflowdoppler; all the procedures were performed immediately. We stratified patients into high, intermediate, or low risk-categories and applied Wells? modified diagnostic model; therefore we used LMWH as protective anticoagulation for up to 72 hours when immediate testing for DVT was not available or for high risk-categories that underwent venography. During a period of 6 months between 15/01/2002 and 15/07/2002 235 patients aged 63 (range 20-95) with clinical suspect of acute DVT came to our attention; 170 patients were managed by the TVP-TEAM and 65 patients were evaluated by the Emergency Department since they arrived in hospital at night or during week ends. Pretest Clinical Probability (PCP) according to Wells score resulted low, moderate and high respectively in 65%, 20% and 15% of patients. Considering a D-Dimer cut off at 500 ng/mL the test resulted negative in 91% and positive in 9%; considering together data on D-Dimers and PCP, D-Dimers were increased in 21% of patients with high PCP, in 4% with moderate PCP and in 5% with low PCP. Colour flow doppler was performed in 99% patients; it revealed 51 (22%) DVT and 17 superficial thrombophlebitis. Among patients with DVT 45 (89%) had a lower limb thrombosis. DVT diagnosis was associated with increased D-dimers only in 24% of patients; regarding to PCP, DVT diagnosis has been made respectively in 56%, 25% and 5% of patients with high, moderate and low PCP. Based on these data showing a very low sensitivity and specificity of D-dimer testing (57% and 20%, even with a negative predictive value of 96%) we recommended a diagnostic strategy combining PCP and colourflowdoppler, until the evaluation of a better cut-off between negative and positive results for D-D assay. Among the 51 patients with DVT, 29% were affected by cancer; in 51% patients the DVT has been defined as idiopathic; 45% were hospitalized for older age or cancer; the mean lenght of hospitalization was 4 days (range 1-40). TEAM TVP data also indicate that home treatment (HT) for DVT is becoming an accepted and safe procedure even in high-risk patients; therefore 43% of patients with DVT and cancer were treated at home with no differences between hospitalized patients and HT in terms of major outcomes. Although an economic analysis was not planned, TEAM TVP approach seems to be cost-effective and well accepted by patients and physicians.

PO145
SAFETY AND EFFICACY OF ENOXAPARIN TREATMENT IN VENOUS THROMBOEMBOLIC DISEASE DURING ACUTE LEUKEMIA
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Venous thromboembolism (VTE) is a quite common complication in the setting of acute leukemia, although the real incidence is so far unknown. Treatment of such complication is still matter of debate, related to a very high bleeding risk in this group of patients. From December 2000 to December 2002, 4 Caucasian patients affected by acute leukemia developed VTE complications: 3 males and 1 female, mean age 55.7 years (range 27-77). Two patients, affected by acute lymphoid leukemia (L1 and L2 according FAB classification), showed deep vein thrombosis during chemotherapy administration; 1 patient affected acute myeloid leukemia (AML, M2 according to FAB) experienced pulmonary thromboembolism at diagnosis, while another patient affected by AML (M4 according to FAB) developed deep venous thrombosis during induction treatment. In all the cases clinical diagnosis of symptomatic VTE manifestations was confirmed by objective imaging procedures: in all cases lower limb venous echo-color doppler, in 1 case ventilation-perfusion lung scan. All the patients were treated with enoxaparin 100 UI/kg twice a day subcutaneously for 3 weeks, followed by a 30% reduction of the total daily dose for about at least 6 months. The same enoxaparin schedule was applied even when platelet count was below 20,000/mL.
Platelet count at the beginning of enoxaparin treatment was very low (mean 50,000/mL, range 12,000-121,000/mL) and the treatment did not affect platelet recovery. During antithrombotic treatment neither VTE recurrences or hemorrhagic complication occurred. Conclusions: enoxaparin revealed to be efficacious and safe in the management of patients affected by acute leukemia complicated by deep vein thrombosis with or without pulmonary embolism. As a matter of fact enoxaparin cured acute vein thrombosis, prevented recurrence and did not cause any hemorrhagic complication despite prolonged severe piastrinopenia.

PO146
PROTHROMBIN A20210 MUTATION AND FV LEIDEN AS POTENTIAL RISK FACTORS OF RECURRENT STROKE IN PATIENTS WITH ATRIAL SEPTAL ABNORMALITIES
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The potential role of patent foramen ovale and atrial septal aneurysm in the genesis of ischaemic stroke (IS) in young adults while taking antiplatelet drugs has frequently reported (Myay et al. N Engl J Med 2001; 345:1740). Until now, however, to uncertainty about the mechanisms of IS systematically recorded, the precise data on the risk of IS recurrence as well as a wide coagulation prophile are lacking despite the clear base-line vascular studies. These latter aspects are of clinical relevance to better establish therapeutic decisions in patients with cardiac septal abnormalities. We here report that the coagulation study for inherited or acquired thrombophilia must also be performed to define better the genesis of the recurrent stroke in these young adults. 13 patients (7F/6M aged 16-44 yrs) with a high risk of recurrent stroke while taking aspirin were investigated for free-protein S (PS), protein C (PC), antithrombin III (ATIII) and lupus anticoagulant (LAC) by automated methods (Dade Behring). Antiphospholipid antibodies (APA) were determined (ELISA assay). ProC global (Dade Behring) as a suitable coagulation test for the determination of the anticoagulatory capacity of the PC system was performed. Prothrombin A20210 mutation and Factor V Leiden were done by polymerase chain reaction and restricted enzyme digestion according to our laboratory procedure. 12-lead electrocardiography, echocardiography, cerebral computed tomography or magnetic resonance imaging, cervical and transcranial ultrasonography were reviewed by neuroradiologists and cardiologists before our study. In all of them, APA and LAC were absent. Two patients showed inherited deficit of PS and one of PC. In 10/13 patients the ProC global normalized ratio was 0.72±0.09 vs 0.93±0.11 in controls (p<0.001), thus suggesting a deficiency (hereditary or acquired) of the PC system. In 5 of them (3 females and 2 males) we found prothrombin A20210 mutation and in 2 (1 female and 1 male) FV Leiden was present. Therefore, in our subjects while taking aspirin the septal disorder is significantly associated with an increased risk of recurrent stroke. A genetic thrombofilic status was found. In our opinion, these patients with documented hypercoagulation and/or inherited thrombophilic disorders, would benefit only from combined more aggressive therapeutic strategies, such as a combination of antiplatelet drugs, long-term anticoagulation, or closure of the foramen ovale.

PO147
TREATMENT OF IDIOPATHIC THROMBOCYTOPENIC PURPURA: RESULTS OF A RANDOMIZED TRIAL COMPARING LOW VERSUS HIGH DOSES OF PREDNISONE AS FIRST-LINE THERAPY
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Ninety-eight adult patients affected by previously untreated idiopathic thrombocytopenic purpura (ITP) (females: 70, males: 28; mean age 38 years, range 20-65), were enrolled in a protocol of study with the aim to compare the efficacy of low versus high doses of prednisone as first-line therapy. Enrolled patients had platelet count <20×10⁹/L or >20×10⁹/L with bleeding symptoms; they were randomised according to the following schedules: a) Random A (47 patients): Prednisone 0.25 mg/kg for 21 days; b) Random B (51 patients): Prednisone 2 mg/kg for 21 days. Response was evaluated at the 3rd week and at the last follow-up. Criteria were as follows: complete response (CR): platelet count >150×10⁹/L; partial response (PR) 50<150×10⁹/L; minimal response (MR): platelet count >20<50×10⁹/L; no response: platelet count <20×10⁹/L. After induction therapy, patients who obtained CR, tapered and stopped prednisone within a week; patients who obtained PR, MR, and patients who did not respond tapered prednisone to 0.10-0.25 mg/kg per day as maintenance therapy. At the 3rd week responses were as follows: Random A (evaluable patients 45): CR 4/45 (8.9%); PR 8/45 (17.8%); MR 21/45 (46.6%); NR 12/45 (26.7%); Random B (evaluable patients 48): CR 16/48 (33.3%); PR 18/48 (37.5%); MR 7/48 (14.6%); NR 7/48 (14.6%). At the last assessment the situation was: Random A (evaluable patients 45): CR 8/45 (17.7%); PR 9/45 (20%); MR 3/45 (6.6%); NR 1/45 (2.2%).
(2.2%) after a median follow-up of 26.2 months (range 1.6-92.8 months); 18/33 (54.5%) responding patients (CR+PR+MR) relapsed after a median time of 12.6 months (range 0.6-67.2 months); 5 patients performed splenectomy (4 NR, 1 M R); 1 patient developed other pathology (La syndrome). Random B (evaluable patients 47): CR 9/47 (19.1%); PR 8/47 (17%); MR 1/47 (2.1%); NR 1/47 (2.1%) after a median follow-up of 28.5 months (range 4.6-99 months); 21/41 (51.2%) responding patients relapsed after a median follow-up of 4.6 months (range 0.86-65.6 months); 5 patients performed splenectomy (4 NR, 1 M R); 2 patients developed other pathologies (La syndrome). Conclusions. These data show that the two therapeutic regimens are comparable in term of efficacy because there are no statistically significant differences comparing the overall responses at the 3rd week and at the last control (p > 0.05). Anyway we can observe that between random A and B there is a difference in quality of response: after induction period, high doses prednisone allow to obtain more CR+PR as compared to low dose (p=0.001). Moreover, number of relapses and splenectomies is the same in the two arms of protocol. Median time at which relapses occurred was shorter in Random B as compared to Random A.

PO148
THE ROLE OF PLATELET APOPTOSIS AND DENDRITIC CELLS IN IDIOPATHIC THROMBOCYTOPENIC PURPURA: A FURTHER CHARACTERIZATION
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The pathogenesis of chronic immune thrombocytopenic purpura (ITP) has not yet been elucidated. Studies suggest that, in particular circumstances, cells dying by apoptosis may trigger a specific immune response. Evidence is accumulating that modifications of autoantigens during apoptosis lead to the development of autoantibodies by bypassing the normal mechanisms of tolerance. Recent studies suggest that also platelets may undergo an apoptotic program. Furthermore, several findings suggest that dendritic cells (DC) are likely to have a role in the pathogenesis of autoimmunity. However, their role as antigen presenting cells in ITP is far to be defined. Therefore, in this study, we investigated the apoptotic platelet program and the immune response to fresh and aged platelets of active ITP (9 cases). Patients, 3 males and 6 females, were at diagnosis (5 cases) or at least 3 months from therapy (4 cases). The median platelet number at the time of the study was 31x10^9/L (range 5-65). After isolation, fresh washed platelets and platelets aged in a serum-free buffer for 3 and 6 days at 37°C were assessed by flow cytometry for phosphatidylserine exposure using annexin-V FITC. The exposure of phosphatidylserine was significantly higher in fresh washed (p<0.05) and aged platelets (day 3; p<0.03) of ITP patients in comparison with the normal counterpart. In order to test if phosphatidylserine exposure was due to apoptosis or platelet activation we tested also the expression of CD62P in fresh and aged platelets. We found that the CD62P expression had about 1.5 fold increase in ITP or normal aged platelets (days +2,+3) in comparison with the fresh samples. By contrast, annexin V showed a 4-8 fold increase. We monitored also the presence of activated caspases in platelets by means of a fluorescent analogue of z-vad-fmk molecule (z-vad-FTIC) and flow cytometry. Caspase activation was present either in normal or ITP platelets but there was no significant difference between the two groups. To further characterize caspases activation, we tested also if the exposure of phosphatidylserine was a caspase-dependent process. Platelets, aged for 3 and 6 days, were incubated with specific inhibitor of activated caspase 3 and 9 (Z-LEHD and Z-DEVD). We found that the exposure of phosphatidylserine was unaffected by these inhibitors. CD14+ monocytes were selected by high-gradient magnetic separation and differentiated to immature dendritic cells with granulocyto-macrophage colony-stimulating factor and interleukin-4 for 6-7 days and then induced to terminal maturation by the addition of LPS. Dendritic cells (DC), either from ITP patients or from normal subjects, were characterized by concentration, immunophenotyping and ability of presenting fresh and aged platelets (day +3 and +6) to autologous and allogeneic T lymphocytes. After 8 days, the culture contained adherent large cell clusters with the typical morphology of DC. There was a trend toward a higher number of DC/1x10^6 CD14+ cells seeded in ITP patients compared with healthy subjects (p 0.06). Flow cytometric analysis indicated that the gated cells expressed all surface markers of mature DCs (CD83+, CD40+, CD86+, CD80+, HLA-Dr+, CD14+). We found that DC from ITP patients show a higher expression of CD86 (p<0.04) in comparison with the normal counterpart. CD14-derived DC of ITP patients, pulsed with fresh or aged platelets before the addition of maturation stimulus, stimulated more efficiently autologous and allogeneic T-cell proliferation in comparison with the DC from healthy subjects (p<0.05). These data demonstrate that ITP platelets could be more susceptible to apoptosis than the normal counterpart and that platelet apoptosis is, in part, caspases-independent. Furthermore, our results clearly demonstrate that the antigen presentation capacity of DC is increased in ITP.
Symptomatic thrombocytopenia is caused in the majority of the cases by an immune-mediated injury and the most common clinical feature is autoimmune thrombocytopenia (AITP). This syndrome, clinically characterized by thrombocytopenia with normal or increased number of megakaryocytes, can occur as an acute or chronic disease. In case of suspected AITP, the first question is to confirm the immunological pathogenesis by detecting the presence of platelet-associated immunoglobulins (PAIg) and demonstrating that these are really specific anti-platelet autoantibodies. However, a large amount of PAIg may be detected on platelets of healthy subjects and of patients with non-immune thrombocytopenia. We studied 42 patients (14 children, 7 pregnant women, 21 adults) with suspected AITP. The detection of PAIg was carried out with a solid-phase test, PSIFT (platelet suspension immuno-fluorescence test) and MAIPA (monoclonal antibody immobilization of platelet antigens). All samples resulted positive to the screening. To differentiate unspecific platelet-bound IgG from specific autoantibodies and to characterize their specific antigenic target, the acid elution test was performed; the autoantibodies obtained were tested with a panel of typed platelets. The elution test was not performed in 12 patients (4 children, 1 pregnant woman, 7 adults) because of insufficient samples. We analyzed 30 eluates with three different methods: the Western blotting technique (using a chumiluminescent marker), a commercial ELISA test (Pak-Auto GTI) and an indirect MAIPA. Four of 30 eluates (2 children and 2 adults) were non-reactive. In the other 26 tests, the revealed antibody specificities were the following: Gplla, Gpllb, Gp120, GplV, Gpla, Gplla, Gplb. Moreover, in some cases, different autoantibodies were associated and/or panagglutinatin. In conclusion, our results confirm in AITP: 1) the relevance of performing the acid eluate test to identify anti-platelet autoantibodies; 2) the targets of autoantibodies are public platelet antigens; 3) autoantibodies reveal a heterogeneous and complex antigenic specificity.
Idiopathic thrombocytopenic purpura (ITP) is an acquired disease characterized by low platelet count caused by autoreactive antibodies that bind to platelets and shorten their life. In adult, ITP resolves spontaneously only rarely. In all symptomatic patients initial therapy with steroid is indicated; response rate varies between 60-90%, depending on intensity and duration of the treatment. Most adults relapse when prednisone is tapered; in these patients splenectomy is the second choice treatment. Since 1988, in our Institution we have treated 1500 patients affected by ITP; in 53 cases (3.5%) we have indicated splenectomy for refractory or relapsed disease. Mean age at splenectomy was 30 years (range 12-76) and male to female ratio was 1:2.5. Mean time from diagnosis to splenectomy was 53 months (range 6-120), with a median of 18 months. All patients had been pretreated with a mean of 3 lines (range 1-6), always including steroids and often including danazol, i.v. IgG, azathioprine. We used i.v. IgG to induce a transient remission before surgery only in a few patients with steroid-resistant severe thrombocytopenia. A few surgical complication occurred but no intra or postoperative hemorrhage. In the most recent years, splenectomy has been performed with a video-laparoscopic technique, which further reduces intra- and post-operative complications. After splenectomy, 15 patients (28%) relapsed; 12 of them were retreated with steroid or danazole and at present are in CR, 7 still under treatment. Two patients refractory to additional therapy (steroid, danazole and chemotherapy) have been treated with rituximab, attaining a second CR. One patient refractory to splenectomy refused additional therapy although having severe thrombocytopenia. All patient relapsing after splenectomy had been steroid resistant before surgery. All patients have been followed up after splenectomy for a mean time of 62 months (range 12-254). 15/52 patients were lost to follow-up after 3 years. During the follow-up, one patient died for intracranial hemorrhage. No other hemorrhagic accident neither thrombotic complication has been observe. Before surgery 6/53 patients (11%) were immunized with Streptococcus pneumoniae vaccine even in the not-vaccinated group, no patient experienced severe infectious complications during the follow-up. Splenectomy is curative in a high percentage of steroid refractory or relapsed ITP patients, and in our series was not associated with complications, even after a long follow-up.
LYMPHOPROLIFERATIVE SYNDROMES II

PO152
ACTIVATED T LYMPHOCYTES EXERT AN ANTI-TUMORAL EFFECT AGAINST AUTOLOGOUS CHRONIC LYMPHATIC LEUKEMIA CELLS
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Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in the Western world. Several phenotypic and multiple functional abnormalities such as depressed response to mitogens and antigens, a diminished mixed lymphocyte reaction and a reduced T-lymphocyte colony forming capacity have been described. Recent in vitro studies have shown that when activated with OKT3 and interleukin-2 (IL-2), T lymphocytes lyse autologous leukemia cells from some patients with CLL. Blood samples from 32 patients with CLL were processed with two E-rosetting steps (day 0) and the T cell fraction was activated with OKT3. After culture in complete medium with IL-2 for 10 days, T-cells were tested for cytotoxicity against autologous B cells with the 51chromium release assay. At the day 0 the T-lymphocyte subsets composition were as follow (median-range): CD3 83% (52.1-95.4); CD4 49.1% (13.2-65.7); CD8 26.5% (8.45-54.8); CD20 2.07% (0.04-30.52); CD56 11.5% (2.25-22.5). After 10 days culture we observed CD3 86% (29.7-94.9); CD4 26.6% (7.1-64.2); CD8 51.6% (15.1-68.3); CD20 0.43% (0.01-4.4), CD56 11.5 (7.1-24.2). The 51chromium release assay showed a cytotoxic index > 30% in 5/32 cases (15.6%). To verify their in vivo effect three groups of NOD/SCID mice were injected intraperitoneally with cells obtained at different stages of the above procedure. Group 1: 100 × 10⁶ CLL B-lymphocytes; Group 2: 100 × 10⁶ CLL B lymphocytes and 20 × 10⁶ non-activated autologous T-lymphocytes; Group 3: 100 × 10⁶ CLL B lymphocytes and 20 × 10⁶ cytotoxic (according to the 51chromium release assay) OKT3 activated autologous T-lymphocytes. Engraftment was assessed on day +30 by means of FACS analysis of peritoneal fluid samples. We found a different rates of B-CLL engraftment in the different set of experiments, probably due to the different stage of disease. The table below shows the results (median - range). To verify their in vivo effect three groups of NOD/SCID mice were injected intraperitoneally with cells obtained at different stages of the above procedure. Group 1: 100 × 10⁶ CLL B-lymphocytes; Group 2: 100 × 10⁶ CLL B lymphocytes and 20 × 10⁶ non-activated autologous T-lymphocytes; Group 3: 100 × 10⁶ CLL B lymphocytes and 20 × 10⁶ cytotoxic (according to the 51chromium release assay) OKT3 activated autologous T-lymphocytes. Engraftment was assessed on day +30 by means of FACS analysis of peritoneal fluid samples. We found a different rates of B-CLL engraftment in the different set of experiments, probably due to the different stage of disease. The table below shows the results (median - range). As expected when mice were injected with non cytotoxic/activated T cells we didn’t find any in vivo antitumor effect. PCR analysis for IgH genes was performed on the different sets of experiment. As expected when we used non citotoxic T lymphocytes, PCR analysis revealed the presence of the rearrangement in all groups of mice, indicating the failure on the eradication of B-CLL leukemia engraftment by the non citotoxic autologous T activated lymphocytes. Viceversa, when we used citotoxic T lymphocytes, PCR analysis showed the disappearance of neoplastic cells and the presence of the only CD3⁺ cells, demonstrating the capacity of the CD3⁺ autologous activated citotoxic cells in preventing the engraftment of BCLL cells. Our results show that it is possible to separate T-lymphocyte from CLL patients on large scale; these T lymphocytes can be cultivated obtaining a final population virtually containing no CD20⁺ cells; in same examined cases, T lymphocytes exert an in vitro cytotoxic effect on leukemia cells; OKT3/IL-2 activated T lymphocytes prevent CLL in the human/mouse chimera, showing that an antitumoral effect occurs in vivo.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD3 (%)</th>
<th>CD20 (%)</th>
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<tbody>
<tr>
<td>I</td>
<td>0.01 (0.63)</td>
<td>5.1 (1.20-10.7)</td>
</tr>
<tr>
<td>II</td>
<td>2.86 (0.47-15.4)</td>
<td>5.24 (2.74-11.1)</td>
</tr>
<tr>
<td>III</td>
<td>10.14 (4.12-17.9)</td>
<td>0.13 (0.05-0.02)</td>
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PO153
TECHNETIUM-99M SESTAMIBI SCINTIGRAPHY IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHIES: A MULTICENTER STUDY ON 154 PATIENTS
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Introduction. Technetium 99m sestamibi (Tc99m sestamibi) is a reliable positive tracer of skeletal lesions in some neoplastic tissues, including multiple myeloma (MM). We investigated the diagnostic role and limits of this procedure in tracing active disease in patients affected by MM or monoclonal gammopathies of undefined significance (M GUS) and compared with those of conventional X-ray. Design and Methods. One hundred
and fifty-four patients affected by MM (129) or MGUS (25) were studied by whole body scans obtained 20 minutes after administration of 740 MBq of Tc99m sestamibi. The clinical characteristics of patients are summarized in Table 1.

Table 1. Clinical characteristics of 154 patients with either MM or MGUS at the time of baseline study.

<table>
<thead>
<tr>
<th></th>
<th>MM (129)</th>
<th>MGUS (25)</th>
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<tbody>
<tr>
<td>Males/females</td>
<td>60 (46.5)/69 (53.8)</td>
<td>14 (56)/11 (44)</td>
</tr>
<tr>
<td>Age (years) median (range)</td>
<td>65 (55-80)</td>
<td>63 (50-74)</td>
</tr>
<tr>
<td>Diagnosis multiple myeloma</td>
<td>111 (86)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>smouldering myeloma</td>
<td>10 (7.8)</td>
<td>28 (8)</td>
</tr>
<tr>
<td>solitary myeloma</td>
<td>8 (6.2)</td>
<td></td>
</tr>
<tr>
<td>Disease stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>25 (22.5)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>18 (16.2)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>68 (61.3)</td>
<td></td>
</tr>
<tr>
<td>M/C type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>91 (70.5)</td>
<td></td>
</tr>
<tr>
<td>IgA</td>
<td>19 (14.7)</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
<td>Bence-Jones</td>
<td>15 (11.6)</td>
<td></td>
</tr>
<tr>
<td>not secretory</td>
<td>4 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Status disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>27 (20.9)</td>
<td></td>
</tr>
<tr>
<td>Complete Response</td>
<td>10 (7.8)</td>
<td></td>
</tr>
<tr>
<td>Partial Response</td>
<td>21 (16.3)</td>
<td></td>
</tr>
<tr>
<td>Minimal Response</td>
<td>2 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>33 (26.5)</td>
<td></td>
</tr>
<tr>
<td>Relapsed</td>
<td>36 (28.7)</td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL) median (range)</td>
<td>11.9 (7.9-16.1)</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dL) median (range)</td>
<td>0.4 (0.01-2.9)</td>
<td></td>
</tr>
<tr>
<td>β2-microglobulin (mg/dL) median (range)</td>
<td>1.3 (0.01-3.2)</td>
<td></td>
</tr>
</tbody>
</table>

A Tc99m sestamibi uptake pattern and/or semiquantitative score was used and scintigraphic findings were correlated with clinical and laboratory data. According to the results of the conventional staging procedure and clinical status two groups of patients were defined: 1) patients with evolutive disease (relapsed, de novo MM in stage II and III, stable disease SD and minimal response MR), 2) patients with not evolutive disease (MGUS, complete response CR, de novo stage I MM, partial response PR). Results. Only one patient (4%) with MGUS showed a positive Tc99m sestamibi scan. X-ray was false positive in 4 cases (16%). The specificity of Tc99m sestamibi in this group of patients was 96% whereas the X-ray specificity was lower (83%). Among the 129 MM patients, a positive Tc99m sestamibi scan and X-ray was exhibited by 60 (47%) and 59 (50%) patients, respectively. The Tc99m sestamibi was positive in 3 patients (30%) with smouldering myeloma whereas X-ray was negative. In solitary myeloma, 6 (75%) and 4 (50%) patients exhibited a positive Tc99m sestamibi scan and X-ray, respectively. Table 2 shows the results of Tc99m sestamibi and X-ray in MM patients according to response to the treatment. The sensitivity and specificity of Tc99m sestamibi in MM was 66% and 73%, respectively. The X-ray sensitivity and specificity was 70% and 64%. Among patients with CR, the Tc99m sestamibi specificity was 90% vs 20% with X-ray. At univariate analysis, the Tc99m sestamibi correlated with disease activity as determined by diagnosis (MM vs smouldering vs solitary myeloma; p = 0.0004), β2 microglobulin (p = 0.007), CRP (p = 0.01), hemoglobin levels (p < 0.001).

Table 2. Results of Tc 99m sestamibi scan and X-ray in patients with MM.

<table>
<thead>
<tr>
<th>Response</th>
<th>Tc99m</th>
<th>X-ray</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (10.0)</td>
<td>9 (90)</td>
<td>0.009</td>
</tr>
<tr>
<td>Negative</td>
<td>8 (80)</td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>Partial Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>7 (33)</td>
<td>14 (67)</td>
<td>0.009</td>
</tr>
<tr>
<td>Negative</td>
<td>14 (70)</td>
<td>6 (30)</td>
<td></td>
</tr>
<tr>
<td>Stable Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (37)</td>
<td>15 (63)</td>
<td>0.009</td>
</tr>
<tr>
<td>Negative</td>
<td>12 (57)</td>
<td>9 (43)</td>
<td></td>
</tr>
<tr>
<td>Minimal Response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>0.009</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>2 (100)</td>
<td></td>
</tr>
<tr>
<td>Relapsed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>25 (71)</td>
<td>10 (29)</td>
<td>0.009</td>
</tr>
<tr>
<td>Negative</td>
<td>20 (69)</td>
<td>9 (31)</td>
<td></td>
</tr>
</tbody>
</table>

ESR (p=0.009), bone pain (p=0.001), bone marrow infiltration (p=0.02), immunoglobulin value (p=0.002) and with status of disease (evolutive vs not-evolutive; p < 0.001). The Tc99m sestamibi scan correlated with response to the treatment (CR vs not-CR; v=0.002) and X-ray bone survey (p=0.01). We found no correlation between positive Tc99m sestamibi and age, gender, stage and M/C-type. At multivariate analysis a positive pattern correlated with evolutive disease (p<0.001). In 53 MM patients the scans were graded according to extension (E) and intensity (I) (Pace’s score system) and were correlated to the most relevant clinical and hematological variables. We found a statistical significant correlation between the pattern score and bone marrow infiltration (p=0.03), β2 microglobulin (p=0.03), bone pain (p=0.003), response to the treatment (p=0.005) and with status of the disease (p=0.01). Conclusion. This study provides evidence that Tc99m sestamibi scan is a useful adjunct to the investigation of MM and MGUS with high specificity and a good sensitivity. The Tc99m sestamibi specificity rose to 100% and 90% in patients with MGUS and MM in CR, respec-
A number of genes are involved in chromosomal translocations in acute lymphoblastic leukemia (ALL). The resulting hybrid genes encode for a host of fusion proteins with roles in key cellular processes, such as cell proliferation and apoptosis. Molecular analysis can provide useful data for both prognostic and therapeutic purposes. We have studied chromosomal rearrangements in 45 cases of ALL. Translocations t(1;19), t(9;22) P210, t(9;22) P190, were analyzed according to the BIOMED 1 protocol, a standardized protocol provided with a confirmation test for first diagnosis, while translocations t(6;11), t(9;11) and t(11;19) have been investigated according to a protocol provided by the Institutes for Pharmacetical Biology JWG University of Frankfurt. The t(4;11) rearrangement was studied with both methods. The lymphoblastoid cell lines SEM and RS, MV, established from two pediatric patients positive for the t(4;11) have been used as positive controls. None of the 45 patients analyzed was found positive for the t(4;11), t(4;11), t(6;11), t (9;11) and t(11;19) translocations. The BCR/ABL gene rearrangement was present in 6 cases (6/45, 11.1%) and of these, 3 cases (6.6%) were p210 and 2 (4.4%) were p190. Overall, the translocations t(6;11), t(9;11) and t(11;19) have been highly specific for the translocations t(1;19), t(4;11), P210, t(9;22) and for those rearrangements that involve the MLL (mixed lineage lymphoma) gene on 11q23, namely t(4;11),t(6;11),t(9;11) t(11;19). It is known, however, that MLL can fuse to over 50 different partner genes in ALL. A new method based on reverse PCR allows for the analysis of such rare variants. This new method offers the advantage of using DNA, instead of cDNA, as template for PCR, thus allowing working with more stable samples and requiring minimum amounts of starting material at the time of first diagnosis. The method uses genomic DNA, digested with restriction enzymes BamHI and BglII and then ligated and amplified by PCR with the Expand Plus long PCR kit (Roche diagnostics). Taken together the three methods can identify the complete spectrum of molecular alterations found in B-ALL.

We report on in 12 adults with bcr/abl-positive ALL, treated with imatinib mesylate in combination with intrahepatic prophylaxis, in which MRD monitoring by qRT-PCR was performed and used in order to modify, when possible, the treatment program. Two patients received Imatinib as maintenance therapy while in 1st CR. One of them is still in 1st molecular CR after 11 months of therapy. The other patient experienced a molecular relapse after 7 months of therapy with imatinib and was then submitted to an allogeneic-SCT from VUD. Eight patients were treated with Imatinib while in relapse (5 in 1st and 2 in 2nd relapse) or being refractory (1). Five out of eight (62.5%) obtained a CHR, 1/8 (12.5%) a marrow-CR with incomplete platelets recovery, and 2/8 (25%) were refractory. Their median response duration was 7.5 months (range, 4-37 months). The median overall survival was 9 months (range, 3-38 months). Four out of six patients relapsed after 4, 7, 8, and 8 months, respectively. In one case we administered imatinib plus α-interferon. After 1 month of therapy, the patient achieved a 3rd molecular CR until now persistent, with a follow up of 37 months. One patient is in CHR after 4 months of imatinib therapy. One patient experienced a molecular relapse after 4 months of therapy at 600 mg/day and then, not being immediately suitable an HLA-matched donor, the imatinib dose was increased up to 800 mg/day. After 4 weeks, he achieved a new molecular remission. Then, he was submitted to allogeenic-SCT from VUD when in molecular CR, 8 months after receiving the first dose of imatinib. Two patients were treated at diagnosis (UPN 11, 12). Both achieved a CHR. One of them relapsed after 4 months, was resistant to further conventional

**Poster Title:** Laboratory Work-Up for a Complete Molecular Diagnosis on ALL Patients; Experience of the U.O. Ematologia ‘A. Businco’, Cagliari

**Authors:** Zucca MG, Biggio V, Angelucci E

**Affiliations:** Ospedale Oncologico A. Businco, Asl 8; Dipartimento di Citomorfologia, Università agli Studi di Cagliari, Italy

**Abstract:** A number of genes are involved in chromosomal translocations in acute lymphoblastic leukemia (ALL). The resulting hybrid genes encode for a host of fusion proteins with roles in key cellular processes, such as cell proliferation and apoptosis. Molecular analysis can provide useful data for both prognostic and therapeutic purposes. We have studied chromosomal rearrangements in 45 cases of ALL. Translocations t(1;19), t(9;22) P210, t(9;22) P190, were analyzed according to the BIOMED 1 protocol, a standardized protocol provided with a confirmation test for first diagnosis, while translocations t(6;11), t(9;11) and t(11;19) have been investigated according to a protocol provided by the Institutes for Pharmacetical Biology JWG University of Frankfurt. The t(4;11) rearrangement was studied with both methods. The lymphoblastoid cell lines SEM and RS, MV, established from two pediatric patients positive for the t(4;11) have been used as positive controls. None of the 45 patients analyzed was found positive for the t(4;11), t(4;11), t(6;11), t (9;11) and t(11;19) translocations. The BCR/ABL gene rearrangement was present in 6 cases (6/45, 11.1%) and of these, 3 cases (6.6%) were p210 and 2 (4.4%) were p190. Overall, the translocations t(6;11), t(9;11) and t(11;19) have been highly specific for the translocations t(1;19), t(4;11), P210, t(9;22) and for those rearrangements that involve the MLL (mixed lineage lymphoma) gene on 11q23, namely t(4;11),t(6;11),t(9;11) t(11;19). It is known, however, that MLL can fuse to over 50 different partner genes in ALL. A new method based on reverse PCR allows for the analysis of such rare variants. This new method offers the advantage of using DNA, instead of cDNA, as template for PCR, thus allowing working with more stable samples and requiring minimum amounts of starting material at the time of first diagnosis. The method uses genomic DNA, digested with restriction enzymes BamHI and BglII and then ligated and amplified by PCR with the Expand Plus long PCR kit (Roche diagnostics). Taken together the three methods can identify the complete spectrum of molecular alterations found in B-ALL.

We report on in 12 adults with bcr/abl-positive ALL, treated with imatinib mesylate in combination with intrahepatic prophylaxis, in which MRD monitoring by qRT-PCR was performed and used in order to modify, when possible, the treatment program. Two patients received Imatinib as maintenance therapy while in 1st CR. One of them is still in 1st molecular CR after 11 months of therapy. The other patient experienced a molecular relapse after 7 months of therapy with imatinib and was then submitted to an allogeneic-SCT from VUD. Eight patients were treated with Imatinib while in relapse (5 in 1st and 2 in 2nd relapse) or being refractory (1). Five out of eight (62.5%) obtained a CHR, 1/8 (12.5%) a marrow-CR with incomplete platelets recovery, and 2/8 (25%) were refractory. Their median response duration was 7.5 months (range, 4-37 months). The median overall survival was 9 months (range, 3-38 months). Four out of six patients relapsed after 4, 7, 8, and 8 months, respectively. In one case we administered imatinib plus α-interferon. After 1 month of therapy, the patient achieved a 3rd molecular CR until now persistent, with a follow up of 37 months. One patient is in CHR after 4 months of imatinib therapy. One patient experienced a molecular relapse after 4 months of therapy at 600 mg/day and then, not being immediately suitable an HLA-matched donor, the imatinib dose was increased up to 800 mg/day. After 4 weeks, he achieved a new molecular remission. Then, he was submitted to allogeenic-SCT from VUD when in molecular CR, 8 months after receiving the first dose of imatinib. Two patients were treated at diagnosis (UPN 11, 12). Both achieved a CHR. One of them relapsed after 4 months, was resistant to further conventional
salvage treatments and died after 5 months. The other
is in CHR, while on therapy with imatinib, with a fol-
low up of 4 months. All the patients who relapsed
presented with bone marrow involvement at relapse; none
presented with CNS involvement, or with other signs of
extramedullary leukemia. Imatinib is effective in
bcr/abl-positive ALL. Unfortunately the duration of
response is brief. The association with intrathecal pro-
phylaxis is safe and effective. Finally, MDR monitoring
by quantitative RT-PCR is useful for an optimal pa-

ten management.
Funding: supported in part by AIL (Bologna and
Pesaro), AIRC, COFIN 2001 (S. Tura) and COFIN 2002 (M.
Baccarani) grants, Ateneo 60% (Baccarani and Facchini),
Fondazione del Monte di Bologna e Ravenna.

PO156
DOMINANT PROGNOSTIC EFFECT OF RISK CLASS OVER TREATMENT
INTENSIFICATION WITH MARROW AUTOGRAFT AND POST-GRAFT
CHEMOTHERAPY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA:
LONG-TERM UPDATE OF THE "IVAP" TRIAL
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Pogliani EM,* Lambertenghi Deliliers G,* Oldani E,*
Barbui T*;
Hematology, Hospital Bergamo, Vicenza, Brescia,
Monza, Milan, Italy

Between 1991-1993, 96 patients were enrolled into
the collaborative IVAP trial, introducing idarubicin for
remission induction and consolidation for adult ALL, and
increasing consolidation intensity with an autologous
bone marrow consolidation (ABMT) phase (pts. aged <51
years) followed by further chemotherapy for 12 weeks
and low-dose maintenance for 6 months (ABMT pts.) or
18 months. Here we provide a long-term update of study
results (Br J Haematol 1999;104:755) with special
emphasis on the results of the ABMT plus chemothera-
py phase in different risk classes. Due to toxicity reasons,
the response rate to induction chemotherapy with IVAP
(idarubicin-VCR-ASP-PDN) was 44% (7/16) with IVAP-
1 (cumulative idarubicin 36 mg/m², concurrent ASP) vs.
90% (72/80) with IVAP-2 (cumulative idarubicin 20
mg/m², delayed ASP), in patients aged 15-60 years
(median 32). 10 patients were electively submitted to an
allogeneic SCT. The ABMT was unpurged and followed
early intensive consolidation with idarubicin-VCR-
CYCLO, CNS radio-chemoprophylaxis, and conditioning
with high dose BCNU-etoposide-melphalan. Multivari-
ate prognostic analysis identified the following risk classes:
standard risk (SR n=26, no risk factor; blast count >25×10⁹/L, B-mature or T-cell phenotype, t(9;22)
or t(4;11)), intermediate-high risk (IHR n=35, any one
risk factor present), and very high-risk (VHR n=18, any
two risk factors). DFS probability at 10 years was 0.30,
and it was 0.29 for ABMT-T treated patients. DFS proba-
bility for SR group was 0.53, 0.27 for IHR group, and
0.06 for VHR group (p=0.0000). To determine further the
impact of ABMT-T plus-chemotherapy phase in the dif-
ferent risk classes, we compared the long-term outcome
of distinct risk groups by ABMT (plus post-graft
chemotherapy) having been carried out or not (the
analysis was not applicable to VHR group). 8-10-year
DFS rates were the following: SR ABMT+ (n=12) 0.56, SR
ABMT- (n=12) 0.41; IHR ABMT+ (n=16) 0.19, IHR ABMT-
(n=10) 0.10. All p values from comparative DFS analyses
were non-significant, particularly when considering
the younger age of ABMT T+ patients (<51 years). In sum-
mary, compared to recently published chemotherapy
series (including the most recent developments from our
group), this long-term update do not support a key role
for a drug conditioning-based ABMT T in adult ALL.

PO157
LONG-TERM SURVIVAL AFTER CHEMOTHERAPY OF ADULT PATIENTS
WITH ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTER
EXPERIENCE OF 112 CONSECUTIVE PATIENTS OVER A 14-YEAR
PERIOD
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Hospital, San Giovanni Rotondo, Italy

Few studies on long-term survival (LTS) after
chemotherapy in adult Acute Lymphoblastic Leukemia
(ALL) patients have been reported. In this paper we ana-
lize a single-center experience about the characteris-
tics of 28 adult ALL patients diagnosed in our Depart-
ment of Hematology between 1985 and 1998. One-
hundred and twelve ALL patients, referred to our obser-
vation from 1984 to 1998 and completely treated by
standard chemotherapy, have been reviewed for this
long-term follow-up. All the patients underwent allo-
geanic stem cells or bone marrow transplantation have
been excluded. The patients included in the study
received chemotherapy according to 5 different sched-
ules such as AIEOP 9502 (5 patients), GIMEMA ALL
0183 (33 patients), ALL 0288 (41 patients), ALL 0394 (7
patients) and ALL 0496 (26 patients) protocols. For each
schedule the patients with LTS resulted 2 (40%),
7 (21.2%), 10 (24.4%), 2 (28.6%) and 7 (29.6%), respec-
tively. On the whole, out of 112 evaluable patients, 28
(25%) had a LTS defined as survival in 1st CR greater
than 5 years (median LTS 126 months; range 62-228
months). The median age was 21 years (range 16-56
yrs.). No case had a WBC count greater than
306.5×10⁹/L (median WBC count: 6.5×10⁹/L - range:
1-28). The Male/Female, L2/L1 FAB cytotype and B/T
cell lineage ratio was 17/11, 13/15 and 19/9, respec-
tively. No case was Ph' chromosome or BCL/ABL posi-
vative. Out of 19 patients belonging to B cell lineage there were 14 Common ALL, 4 Early-Pre-B ALL and 1 Pre-B ALL. Among the 9 cases with T cell lineage ALL, 4 case showed an early-T phenotype and 5 case a late-T phenotype. Myeloid antigens such as CD13, CD33 and CD15 were expressed in 7/15 (46.7%), 7/16 (43.7%) and 2/18 (11.1%) evaluable cases, respectively. CD34 antigen at a cut-off greater than 20% was present only in 4/20 (20%) patients. Only one T-ALL patient showed a late neuropsychological sequelae although this patient did not receive prophylactic CNS irradiation. During induction or consolidation chemotherapy 10 patient (35.7%) with LTS had an adjunctive event such as systemic mycosis (2 cases), pulmonary embolism (1 case), intestinal infarction (1 case), interstitial bronchopneumonia (5 cases) and acute appendicitis (1 case). Our single-center report on a long-term survivors setting of adult ALL patients showed that the standard risk degree and the contemporary cytokine storm related to adjunctive events, probably provide a complete disappearance of the minimal residual disease and the potential cure of ALL patients. 

In an ongoing valuation we are investigating the presence of molecular markers Bcl2 or VDJ-IgH in lymphoproliferative disorders with bone marrow involvement before and following the therapy. The value is finalized to disclose the correlation between molecular status and clinical outcome, to see which type of therapy exerts a major efficacy on disappearance of molecular marker and which disease shows a better clearance of molecular marker. Up to date 24 patients are available because they completed the therapeutic program: there are 19 patients with follicular or diffuse centre cell lymphoma (FDCLL), 3 chronic lymphocytic leukemia (CLL) and 2 mantle cell lymphoma (MCL). The treatment of 20 patients consisted of sequential therapy with CHOP, high dose Cyclophosphamide (CTX) and Rituximab, high dose Aracytin and second Rituximab, collection of CD34 cells, PBSCT with BEAM conditioning regimen. The other patients received CHOP plus Rituximab 3 patients and allogeneic bone marrow transplantation one patient. The molecular value is available in all patients following conventional therapy during the sequential program and at the end of therapy. Only 3 patients (FCCL) showed a negative molecular result following the conventional therapy either given alone or in the sequential program, the other 21 patients were still positive. Other 5 patients gained a negative molecular result following the high dose CTX and Rituximab. Other 7 patients went into molecular negativity following the high dose Aracytin and second Rituximab, 4 patients became negative following high dose therapy with BEAM. Five patients never achieved molecular remission; 2 of them were FCCL treated by conventional CHOP plus Rituximab and 3 with CLL treated by sequential therapy. All patients are now alive except one patient who died 5 months following high dose therapy for brain hemorrhage; at the follow up all patients, molecular positive, have clinical evidence of disease, none of those negative relapsed. In conclusion this ongoing and preliminary value seems to demonstrate that the disappearance of molecular marker in lymphoproliferative disorders correlates with the clinical outcome of the disease, it is more difficult to be eliminated by conventional treatment and it is still persistent in CLL despite the high dose treatment. 

PO158 
MOLECULAR VALUE IN LOW GRADE LYMPHOPROLIFERATIVE DISORDERS 
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PO159 
DIAGNOSTIC UTILITY OF FLUORESCENCE IN SITU HYBRIDIZATION IN LYMPHOPROLIFERATIVE DISEASE 
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Fluorescence in situ hybridization (FISH) is a sensitive method for the routine identification of leukemia and lymphoma-associated chromosomal translocation. In order to evaluate the diagnostic utility of FISH in lymphoproliferative disorders we investigated the presence of translocation t(11;14) or t(14;18) on interphase cells from peripheral/bone marrow blood (44 cases) or from cell suspension from lymph node or other pathological tissue biopsies (16 cases) in a series of 60 patients using highly sensitive dual color, dual fusion translocation probes (Vysis). Results were compared with pattern of BCL1-IgH or BCL2-IgH PCR rearrangement. Forty-nine patients with immunophenotype suggestive of mantle cell lymphoma (MCL) or variant chronic lymphocytic leukemia (vCLL: CD5+, CD23+ or ±, SIg bright), were analyzed by FISH for assessment of translocation t(11;14). Twenty-three cases showed a negative concordance, 10 a positive concordance and the 16 discordant cases were all FISH positive for the translocation and PCR negative (PCR specificity=100%, PCR sensitivity=38%). Eleven patients with immunophenotype correlated with a diagnosis of follicular lymphoma (Fo-NHL: CD10+), were analysed by FISH to detect the translocation t(14;18). Three cases showed a negative
concordance with PCR results, 4 cases showed a positive concordance and the 4 discordant cases were all FISH positive for the translocation and PCR negative (PCR specificity=100%; PCR sensitivity=50%). A comparison of detection of BCL1-IgH or BCL2-IgH PCR rearrangement on fresh or paraffin-fixed tissues showed a discordance in 20% of the cases examined. We conclude that FISH assay is very useful in confirming the diagnosis of MCL or Fo-NHL due to higher detection rates than PCR methods. Although FISH is not useful in the follow-up of these lymphoproliferative disorders to detect minimal residual disease, because of its low analytical sensitivity compared to PCR, it should be introduced as a routine assay for a rapid diagnosis of these entities.

PO160  
IMMUNOGLOBULIN VH GenES AND CD38 EXPRESSION ANALYSIS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (B-CLL) cases can be divided into 2 subgroups based on presence or absence of significant numbers of mutations in the variable region of the immunoglobulin heavy chain (IgVH) genes. Patients whose leukemic cells express unmutated IgVH regions often have aggressive disease, whereas patients whose leukemic cells express mutated IgVH regions more often have an indolent disease. However, the clinical usefulness of IgVH gene mutation analysis is offset by the high cost and level of expertise required for this technique. Reports suggest that CLL B-cell CD38 expression may be a surrogate marker of IgVH gene status. Currently, however, the usefulness of this surrogate marker is controversial. Some of the difference may be due to technical aspects of the CD38 assay and the choice of an optimal cut point for the number of CD38+ cells. Various studies considered CLL cases with 30% or more CD38 expression cells to be CD38+, but the largest study to date found that a cutoff of 7% was best at separating different prognostic groups. Design and Methods. In the current study, to further clarify the correlation between VH gene mutation and CD38 expression, we analyzed VH mutation status by polymerase chain reaction and sequencing and CD38 expression by flow cytometry using direct conjugate antibodies (anti-CD19-FITC/anti-CD38-PE) in 42 LLC patients. Results. On the basis of the percentage of CD38 antigen expression, 42 patients were subdivided in three groups: CD38<7% (20 patients), 7%< or = CD38< or = 30% (11 patients) and CD38>30% (11 patients). Using a conventional cutoff of 98% sequence identity to the nearest germ line IgVH sequence, 16 cases (38%) were classified as Ig-mutated CLL and 26 cases (62%) were classified as Ig-unmutated CLL. In the first group only in two cases had unmutated genes, in the second group among 11 patients 5 were unmutated and 6 mutated, in the last group 9 patients were unmutated and 2 mutated (Figure 1).

Figure 1 Correlation between CD38 expression levels and IgVH gene mutation status: Blue color: mutated cases (M, mutated); Yellow color: unmutated cases (G, germline)

Conclusions. In this study, high CD38 expression levels correlated with unmutated VH genes and low CD38 expression levels with mutated IgVH genes. However, as described by others, in approximately 22-23% of patients the CD38 expression levels did not predict whether the VH genes were unmutated or mutated. Particularly intermediate levels of CD38 expression (7%< or = CD38< or = 30%) cannot be used as surrogate marker for VH gene mutational status in CLL.


PO161  
MOLECULAR ANALYSIS OF T CELL RECEPTOR CDR3 CLONOTYPES IN LGL SYNDROME: A POLYCLONAL PATHOPHYSIOLOGY FOR A CLONAL DISEASE

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Large granular lymphocyte (LGL)-syndrome is a clonal lymphoproliferative disorder of cytotoxic T cells. Clinical course of LGL-disease is generally indolent, and often characterized by mono- or multi-lineage cytopenias. LGL syndrome may arise from both NK (CD3-) or T cells (CD3+); in this latter case, clonality can be

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assessed based on the T-cell receptor (TCR)-β chain. Sequencing of the highly variable (and antigen-specific) complementarity determining region 3 (CDR3) represent an unique molecular signature of the leukemic clone (clonotype); we have applied this technique to identify and characterize specific clonotypes in a large cohort of LGL-leukemia patients. Thirty-three patients were analyzed: 3 presented with a pan-cytopenia, 5 with a bi-lineage leukemia, and the remaining 25 showing a mono-lineage cytopenia; their HLA-type was extremely diverse. By flow cytometry, expansions of one or few TCR variable-β (Vβ) subsets were initially identified; CD8 lymphocytes were selected, and Vβ-specific CDR3-pools amplified by RT-PCR from the extracted RNAs. The unique nucleotide and amino acid sequences of leukemic clones were determined by direct sequencing of the Vβ-specific RT-PCR amplicons, or after cloning in bacteria. Most patients showed the expansion of one clone with its own functional TCR, but in some cases 2 different clonotypes were identified. No preferential usage of particular Vβs neither Jβs was demonstrated. Clonotypes were always patient-specific, and protein alignment algorithm failed in demonstrating significant sequence homology. The identified sequences were utilized to design clonotype-specific PCR-primers; using a very sensitive semi-nested PCR amplification, specific bands were detected also in other patients and healthy volunteers. However, sequencing analysis documented that the original clonotypes were restricted to the patient from whom they were first derived, and isolation in different subjects reflects just the presence of highly homologous CDR3 sequences. Specific TCR clonotypes derived from LGL patients can be used as disease molecular marker, useful in monitoring disease course. The presence of a functional, likely antigen-specific, TCR on clonal T-cell subsets expands in LGL leukemia is consistent with the hypothesis that the proliferation is, at least initially, antigen-driven. The high frequency of cytopenia in LGL-disease suggests that a continuum may exist between this extremely clonal disease, and other bone marrow failure syndromes with poly- or oligo-clonal immune pathophysiology, sharing the same hematopoietic target. The antigenic selection suggested by the concomitant presence of more than one clone in the same patient, as well as the evidence of homologous clonotypes (both intra- and inter-patient), support the hypothesis that certain common hematopoietic antigens may trigger the immune system. However, given the extent of HLA-diversity in our cohort of patients, which explains the CDR3-heterogeneity seen, larger collection of clonotypes and structural analysis of CDR3-motifs is still needed to confirm this idea. In summary, we first describe a large database of LGL-specific clonotypes; even if antigenic specificity had not yet been identified, our findings support the idea that LGL-leukemia arise from an antigen-driven (auto)immune-response, and is not a strictly-autonomous malignant proliferation. The hypothesis that the target of such immune attack could be the hematopoietic progenitors would explain disease manifestations but still needs further investigation.

**PO162 INFECTIOUS COMPLICATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: EXPERIENCE OF A SINGLE CENTER**

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The literature provides no specific data concerning the type and the risk factors for infections occurring in adult acute lymphoblastic leukemia (ALL). Therefore, we retrospectively analyzed incidence and factors affecting infectious complications in 97 adult ALL patients that underne conventional chemotherapy during a 14-year period. The median age was 45 years (range 16-79) and 31 patients (32%) aged > 60 years. Forty-three patients (44%) received induction therapy according to GIMEMA ALL 0288 trial and 18 patients (19%) according to GIMEMA ALL 0496 trial; 15 elderly patients (15%) were treated with Daunoxome, vincristine and dexamethasone; 8 patients (8%) were given regimens containing cytarabine and 13 (14%) received other intensive chemotherapies. During induction phase 64 patients (66%) were hospitalized in HEPA filters rooms. Prophylaxis with quinolones was given to 73 patients (75%) and 42 patients (43%) received granulocyte colony-stimulating factor (G-CSF). The duration of neutropenia (ANC < 500/µL) was more than 7 days in 48 patients (49%). Seventy-three out of 97 patients (75%) achieved CR and 10 (10%) had resistant disease. During induction therapy 48 patients (50%) developed fever classified as microbiologically documented infection in 31 patients (65%), as unexplained fever in 12 (25%) and as clinically documented infection (all pneumonia) in 5 patients (10%). Bacteremia was the most common infection (71%), mainly caused by E. Coli (44%) and Pseudomonas aeruginosa (37%). Nine patients (18%) developed pneumonia and 3 aspergillosis were documented. Fourteen patients (14%) died during induction and infections were the cause of death in 11 patients. Age was the only risk factor significantly associated with both infection development and mortality (≤60 years = 42% vs > 60 years = 65%, p = 0.04; ≤ 60 years = 33% vs > 60 years = 68%, p = 0.04, respectively. Considering the 66 patients ≤ 60 years old, antibiotic prophylaxis resulted the only factor affecting infection onset. Among the 31 older patients no factors were significantly associated

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with development of infection; in contrast, death from infection was influenced by chemotherapy regimen (weekly schedule = 67% vs others = 17%, p = 0.03). Of 83 patients who underwent subsequent treatment, 18 (22%) developed fever and 13 (16%) died. Seven patients had fatal pneumonia by CMV (2), Aspergillus spp. (2), Pneumocystis carinii (1) and 2 by unknown agent. Eight patients developed bacteremia (5 fatal), 1 died of invasive candidiasis. No factors influenced development of infection while factor affecting mortality was the induction cumulative dose of corticosteroid (2600 mg = 31% vs > 2600 mg = 69%, p = 0.03). In summary, 50% of patients with ALL who undergone induction therapy develop fever, documented in 75% of cases. Of concern is the occurrence of serious infections during the treatment following induction therapy, mainly caused by opportunistic agents and associated with high mortality. In conclusion, tailored chemotherapies should be given to older patients, antibiotic prophylaxis may be useful in younger during induction chemotherapy and prophylaxis of opportunistic infections should be taken into account during post-remission therapy in patients of any age receiving high-dose corticosteroid during induction therapy.

In normal T-cell development, thymic epithelial cells (TECs) exert an inductive role in migration, survival and maturation of thymocytes through the generation of a local gradient of cytokines and the establishment of adhesive interactions with immature T cells. Interleukin-7 (IL-7), secreted by thymic stromal cells, has a nonredundant role in regulating the early phases of T-cell differentiation. Furthermore, it is known that IL-7 promotes survival and induces cell cycle progression of T-cell acute lymphoblastic leukemia (T-ALL) cells in vitro. In this study we analysed the role of IL-7 on T-ALL blast survival within the microenvironment generated by T-ALL-TEC interaction. To this aim T-ALL blasts derived from 10 adult year-old man, was diagnosed with Ph+ ALL in May 2001. He was admitted at our Division with fever and bilateral epistaxis. Physical examination showed abdominal petechiae and a mild splenomegaly (4 cm). Blood count was: hemoglobin (Hb) 87 g/L, platelets (Plts) 5 × 10^10/L, white blood cells (WBC) 84 × 10^9/L (blasts 92%). A bone marrow biopsy and aspirate were diag-
nastic for an acute lymphoblastic leukemia, FAB subtype L1-L2. Cytogenetic analysis revealed a complex karyotype, with anomalies of chromosomes 1, 3, 13 and presence of Ph chromosome [t(9;22)(q34;q11)], with both P190 (e1a2) and P210 (b3a2) BCR-ABL transcripts. He initially received two courses of chemotherapy (vin.cristine, doxorubicin, L-asparaginase and prednisoni, then high-dose cytarabine and idarubicin), obtaining a complete hematologic and cytogenetic remission (100% 46,XY), but still BCR-ABL-positive. Molecular response was attained after two more courses of therapy with vincristine, Adriamycin, cyclophosphamide and methotrexate. In September 2001, while still in complete remission, he received an allogeneic bone marrow transplant from an HLA-identical brother; conditioning regimen was busulphan + cyclophosphamide (BuCy). He did not develop any acute or chronic graft-versus-host disease (GVHD), despite a progressive reduction of immunosuppressive therapy (cyclomairine A) until suspension after seven months, and maintained a complete hematologic, cytogenetic and molecular response for about 12 months. In September 2002 there was a relapse of ALL; cytogenetic analysis was not evaluable due to lack of metaphases, but RT-PCR detected BCR-ABL translocation, with P210 (b3a2) and P190 (e1a2) rearrangements. He received a course of chemotherapy with liposomal daunorubicin and high-doses cytarabine, obtaining a complete cytological remission but remaining BCR-ABL-positive. At time of hematologic recovery, on October 14th, Imatinib mesylate (Glivec) therapy was started at a daily dose of 400 mg, rapidly increased up to 600 mg/day. After five weeks, Imatinib daily dose was reduced to 300 mg, due to grade III WHO thrombocytopenia. Bone marrow aspirate and molecular analysis for BCR-ABL were performed on a monthly basis, confirming a sustained cytological remission; at the fifth week (November 11th) RT-PCR analysis for BCR-ABL was negative, and this result was confirmed since then. Imatinib was maintained at a reduced daily dose of 300 mg, due to persistent grade II thrombocytopenia, until the sixth month, but therapy was never discontinued. At the time of writing (end of April 2003) the patient is still in complete hematologic and molecular response, with a Glivec dose of 400 mg/day and negligible non-hematologic toxicity. Ph+ ALL is characterized by marked refractoriness to therapy; allogenic SCT is the only curative approach, providing that is performed in first CR. Treatment of relapse is often empirical, since neither chemotherapy, SCT nor Imatinib alone usually grant a durable response. The case we report seems to indicate the efficacy of a combination of intensive chemotherapy and Imatinib. Chemotherapy reduces the bulk of disease and grants a second CR, thus the targeted therapy, started as soon as hematologic recovery is attained, can achieve a molecular remission.

References


and Ph' acute leukemias, the occurrence of B lineage BCR/ABL gene were demonstrated. Among BCR/ABL was present and both p190 and p210 fusion proteins of and acid-phosphatase disappeared. Ph' chromosome diagnosis and age over 75 at diagnosis were significantly more frequent in the second studied decade (Table 1): this let us suppose that at least a part of the observed increase in incidence is apparent, due to an easier access of people to an health care system that, moreover, during studied time acquired better diagnostic efficiency in hematology.

**Table 1. Characteristics at diagnosis.**

<table>
<thead>
<tr>
<th></th>
<th>1974-1983 no. of patients (%)</th>
<th>1984-1993 no. of patients (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 75 years at diagnosis</td>
<td>66/361 (22)</td>
<td>175/521 (33)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Incidental diagnosis*</td>
<td>32/107 (29)</td>
<td>157/268 (58)</td>
<td>0.000001</td>
</tr>
<tr>
<td>Binet Stage A at diagnosis*</td>
<td>37/107 (35)</td>
<td>135/268 (50)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Only patients of Ospedale Oncologico-Cagliari.

CLL presents among Sardinian residents age and sex distributions similar to those reported for other western populations: it is absent in younger people and its incidence increases with increasing age reaching its maximum in the oldest people without plateauing; a male prevalence is consistently present and increases with increasing age. Age standardized rates (World population) in 1984-1993 reached values on the range of those reported in western populations (male:2.88 × 100,000 person years-SE 0.17; female: 1.50 × 100,000 person years-SE 0.11). Expected cases for 2001(calculated by applying age- and sex-specific incidence rates of the last 5 years to expected Sardinian populations at 2001- obtained from ISTAT- age strata by age strata, and by summing the results over the strata) should be 64; of them 62% should be over 65 years of age.
Campath-1H in 12 fludarabine refractory/relapsed B-CLL patients. Clinical characteristics: median age 65 years, median time from diagnosis 7 years; according to Rai stage 9 (75%) patients were stage IV, 1 stage II and 2 stage I; 4 patients had grade 3 performance status (WHO), 5 grade 2 and 3 grade 1. Previous treatments consisted of at least 3 different chemotherapy regimens (median 7 cycles); moreover, 4(33%) patients had been also treated with monoclonal antibody therapy (Rituximab), one of them after relapse from a bone marrow transplant. Treatment: Campath-1H was delivered s.c. onward from week 1 to 3; on day one, 3 mg of the drug was administered in the thigh, if well tolerated, this dose was raised to 10 mg on day 3 and then raised to the target dose of 30 mg split into two injection sites (1.5 ml at each site) on day 5. After the dose-escalation phase, and the disappearance of 'first dose' skin reactions, almost all patients self-administered Campath-1H three times a week for a minimum of 4 to 12 weeks. Prophylactic medication against first-dose reactions included paracetamolo (1 gr orally) and antihistamines (desclorfeniramine 6 mg orally), given 30 minutes before the injections. Anti-infective prophylaxis, acyclovir (400 mg twice daily) and cotrimoxazole (twice daily, 3 times a week) was given during and for 12 weeks after completion of therapy. Patients were considered valuable for response once they received at least 4 weeks of treatment. Results: at June ’03, two patients were ‘too early’ and 10 were valuable for response. Eight patients were alive +6 to +10 months for 12 weeks after Campath-1H therapy, 2 patients died: one due to progressive disease and the other by gastrointestinal bleeding. According to NCI-WG criteria (Cheson et al. Blood 97(12):4990) the overall response rate (OR) was 70%: 3 (30%) were the complete remission (CR), 4 the partial response (PR), 2 patient had a stable disease and one was refractory. Safety: local injection site reactions (erythema/edema) were seen in 2 patients and promptly disappeared usually within 1 week. Transient rigor, fatigue and itching was observed in 3, 2 and 6 patients, respectively. Fever >38°C was registered in 4 patients thus requiring the temporarily interruption of drug administration: 3 were FUO and resolved within 7 days, 1 was a grade II sepsis. Apart from long-lasting lymphocytopenia, which occurred in all patients, the main haematologic toxicity was neutropenia. Five patients had a grade IV neutropenia which promptly resolved with G-CSF injections. Conclusions: we confirm Campath-1H is active in heavily pretreated B-CLL patients. Moreover, subcutaneous administration induce very few first-dose flue-like symptoms and may reduce health care cost in comparison with the intravenous infusion.
cases, apparently non correlated with therapy, although this event should require a more careful evaluation. In conclusion, this preliminary report suggests that oral combination of low-dose Flu and Cy is safe and may induce rapid responses in about 80% of patients with pre-treated CLD. A more adequate number of patients, as well as a longer follow-up, are needed to confirm these encouraging results and better clarify the optimal dosage and schedule of oral Flu.

**Introduction**

Fludarabine is a Th-lymphocyte highly specific purinic analogue used in lymphoproliferative disorders treatment; well known its role in disclosing latent autoimmune B-CLL related diseases. We report the case of a woman affected by B-cellular lymphoproliferative leukemic disease, CD20+, CD22+, CDS+, Smlgκ+, with splenic and bone marrow involvement, who developed severe anemia (G4) and thrombocytopenia (G3) after four months since treatment with Fludarabine. For patient’s religion prohibits transfusions, we gave her high dose steroid inbolus, followed by anti CD-20 monoclonal antibody (Rituximab) administration; the rational of such approach is that Rituximab is effective in eliminating autoreactive CD20+ clones, whether their neoplastic or disimmune origin. Case report: 56 year-old female, affected by B-cellular lymphoproliferative disease, CD20+, CD22+, CDS-, Smlgκ+, with splenic and bone marrow involvement, treated with 6 courses of Fludarabine (30 mg/m2 5 days, each 28), developed very severe anemia in the 5th week; at weeks of treatment hemoglobin and platelets values were 12 g/dL and 120,000/cm3 and the patient remains in CR, without any further therapy. Conclusions: our experience confirms that the treatment with Rituximab: 1) has specific activity against CD20+ lymphoproliferative diseases; 2) is effective in controlling disreactive phenomena, even due to purinic analogues induced autoimmune disorders; 3) is able to achieve a fast and long-lasting response; 4) is safe, even in extreme conditions (Hb 3.6 g/dL).

**PO169**

**RITUXIMAB IN THE IMMUNOLOGICAL TREATMENT OF SEVERE POST-FLUDARABINE CYTOPENIA: A CASE REPORT**


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**Introduction**

On the basis of the efficacy of FAND, a Fludarabine (Flu) combining therapy protocol, and of Campath-1H, a humanized anti-CD52 monoclonal antibody used as a single agent in CLL, we conducted a clinical study to determine the feasibility, safety and efficacy of a FAND/Campath-1H combination schedule given to 7 poor prognosis CLL patients with advanced and previously treated disease. Median age was 50 years, median CLL duration 52 months, median number of prior treatments 3, that in all cases included Flu. Enlarged nodes or spleen were present in all cases. Two patients were refractory to prior Flu. In all cases, the CD52 antigen was expressed by the leukemic cells. The FAND/Campath-1H schedule was administered on an outpatient basis. Patients received two courses of FAND followed by two courses of FAND/Campath-1H combination. FAND included: Flu (25 mg/m2 i.v. daily at 0, 4 and 48 hours) combined with ARA-C (700 mg/m2 i.v. at 4, 28 and 52 hours), Novantrone (10 mg/m2 i.v. at 6 hours) and Dexamethasone (20 mg i.v. daily on days 1 to 3). The third and fourth FAND courses were followed by 3 administrations of 30 mg of Campath-1H (supported by Schering SPA, Italy) i.v. After 4 weeks from the fourth FAND/Campath-1H course, a response assessment was performed and patients with evidence of residual disease received further doses of 30 mg of Campath-1H i.v. 3 times weekly for 4 weeks as postinduction cytoreductive therapy. Infection prophylaxis consisted of fluconazole, acyclovir, trimethoprim/sulfamethoxasole continuing at least 6 months following Campath-1H. G-CSF was given in the presence of severe neutropenia. Quantitative cytomegalovirus (CMV) viremia was performed weekly. A clinical, cytometric and molecular evaluation of response was assessed after 4 weeks from the fourth FAND/Campath-1H course, after the postinduction cytoreductive phase with Campath-1H and, thereafter, every 3 months during the follow-up. At the present
time, the response is evaluable for 4 patients. All patients showed a response with a marked clearance of PB and BM leukemic cells, and the 2 patients with prior adenomegaly showed a greater than 50% reduction of the nodal size. A cytometric CR was documented in 3 patients, associated in 1 also by a molecular remission; the fourth patient with residual enlarged nodes achieved a PR. Infusion related adverse events included fever, rashes and rash at the time of the first administrations. The most commonly observed hematoxicity was represented by grade III-IV neutropenia and thrombocytopenia. All patients showed a profound decrease in T cells (CD3+, CD4+ and CD8+). One case of pneumonia was recorded. Three patients developed a CMV viremia after the first FAND/Campath-1H course and received ganciclovir therapy with clearance of the CMV viremia. In conclusion, our preliminary data on a limited number of patients indicate that the FAND/Campath1-H schedule shows significant activity in poor prognosis, pre-treated CLL patients. The long-term therapeutic benefit and toxicity of this schedule needs to be further investigated.

PO171
GEMCITABINE AND CAMPATH-1H: A TREATMENT PROPOSAL FOR SEZARY SYNDROME
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Sezary syndrome (SS) is a variant of cutaneous T-cell lymphoma with frequent involvement of lymphnodes, bone marrow and peripheral blood. Patients are generally characterized by exfoliating erythroderma, plaques and nodules causing intense itching. Various therapeutic approaches are described for SS (extracorporeal photopheresis, mono- or polychemotherapy, retinoids) with a variable but in any case low response rate. In a majority of cases, responses are short-lasting with refractoriness to further treatment. We present a proposal for SS treatment, using a chemotherapeutic agent as gemcitabine, known for its efficacy in cutaneous lymphomas, in combination with the anti-CD52 monoclonal antibody, Campath-1H, cytotoxic for B and T lymphocyte, and recently used as single agent in a Swedish multicentric clinical trial on SS. A 49-year old male was observed one year ago for generalized exfoliating erythroderma, desquamation, plaques and nodules, particularly at foot soles, with intolerable itching; abnormal cell with typical cerebriform nuclei were observed in peripheral blood; bone marrow cytology and histology showed a significant infiltration by the same cells, having the immunophenotype CD3+, CD2+, CD5+, CD4+. A diagnosis of SS was made. The patient was treated in another institution by extracorporeal photopheresis followed by polychemotherapy (fludarabine, mitoxantrone, cyclophosphamide) without significant benefit. The patient returned to our care with a diagnosis of refractory disease. We designed a two drug regimen with Gemcitabine and Campath-1H. Gemcitabine was administered at a weekly dose of 1200 mg/m2 for three weeks, and was followed after two week interval by subcutaneous injection of Campath-1H at dose of 30 mg/day three times a week for four weeks. This treatment was administered for three successive courses. The potent immunosuppressive effects of Campath-1H required prophylaxis with cotrimoxazole, antifungal and antiviral drugs. At the end of the first course the patient showed complete remission of the cutaneous lesions and disappearance of the cerebriform cells from peripheral blood and bone marrow. During Campath-1H treatment, cytomegalovirus reactivation occurred, which was responsive to specific antiviral therapy. Gemcitabine was administered in day-hospital; Campath-1H, except for the first week, was administered at patient home with good compliance and without local or systemic side effects. If large studies with a prolonged follow-up will confirm these data, a protocol with gemcitabine and Campath-1H could be adopted as first line treatment in this very rare disease.

PO172
CHRONIC MYELOGENOUS LEUKAEMIA AND HODGKIN’S LYMPHOMA IN THE COURSE OF CHRONIC LYMPHOCYTIC LEUKAEMIA: A CASE REPORT
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The coexistence of hematopoietic and lymphoid malignancies unrelated to any treatment is quite rare. We report on the case of a patient with B cell chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML), who subsequently evolved to myeloid blast crisis (BC), and presented lastly with a Hodgkin’s lymphoma. We reported the updated follow-up of a case yet presented in the previous meeting yielded in Turin (Montanaro et al., SIE 1999). In January 1998, a 65-years old male, presenting lymphocytosis, enlarged axillary lymph nodes and mild hepatomegaly, was referred at our attention. The immunophenotyping analysis showed CD 19/CD5 lymphocytes. A diagnosis of CLL was made. Six month later, the white blood cell rose to 90×10⁹/L and a 65% myeloid cells on peripheral blood (PB) were found. A bone marrow (BM) biopsy revealed an increase of myeloid lineages and nodular infiltration by mature CD20/CD5 lymphocytes cells. The
cytogenetic study showed the Ph chromosome t (9; 22) (q34:q11) in all metaphases and the RT-PCR evidenced the hybrid bcr/abl rearrangement (p210 /p190). Southern Blot for JH analysis of DNA from PB and BM samples evidenced a clonally restricted B cell population, demonstrating the distinct origin of the two entities. The patient was treated with hydroxyurea and obtained a hematologic response, remaining well under until April 2002 when the myeloid blastic crisis occurred. He stated imatinib mesylate, 600 mg/day, achieving the karyotypic major and complete response (CR) after three and six months respectively. In November 2002, the patient was re-admitted because of severe malaise and fever. No rash, skin lesions or lymphadenopathy were found. The lungs were clear, the abdomen was soft, the liver and the spleen were not felt and the neurologic examination was not remarkable. X ray of the chest and the laboratory evaluation showed no substantial abnormalities. Specimens of blood, urine and stool, obtained at different times, were negative. The BM re-evaluation showed normal findings. An intravenous broad-spectrum antibiotic therapy was begun. Patients presented further deterioration and the appearance of abdominal and superficial lymphadenopathies. A node biopsy revealed a diagnosis of Hodgkin’s lymphoma. The patient deteriorated progressively and died in January 2003 because of pneumonia. CML, as second malignancy after CLL, has not yet been reported. The role of the impaired immunosurveillance typical of CLL and the existence of a clonal instability may explain the findings recorded in the clinical course of this reported case.

PO173
Not published.

PO174
RITUXIMAB PLUS MODIFIED CEOP ARE EFFECTIVE IN THE TREATMENT OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA
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In large B-cell lymphoma the addition of rituximab (R) to standard- dose CHOP improves response rate and overall survival in elderly patients and such a feature is restricted to those patients who express bcl-2 protein (Blood 2003; 101:4285-9). However, to the best of our knowledges, studies dealing with the association of R and CHOP-like regimens in B-cell chronic lymphocytic leukemia (CLL), a disease characterized by the over-expression of bcl-2 protein, are virtually absent. With this background we have treated 10 B-cell CLL patients with an association of R (373 mg/m², D-1) and a modified CEOP-regimen [Vinblastine 5 mg/m²; Epi-Doxo 50 mg/m²; Cyclophosphamide 750 mg/m² (D-2); PDN 100 mg (D 2-5)]. There were 7 males and 3 females and median age was 67.5 years (range, 61-75). The median number of previous lines of therapies was 2 (range, 1-3). All patients had previously received chlorambucil, 5 fludarabine (F), 4 CHOP-like regimens and one patients R+FC. According to status of disease, as assessed at the time of inclusion in the present study, 7 patients patients displayed a relapsed chemosensitive condition and 3 a refractory one. In addition, 6 were in Binet stage C and 4 in B; median lymphocyte count being 90.4x10^9/L (range, 1.9-170). Either cotrimoxazole prophylaxis or filgrastim (Sug/kg/day) from day +5 to +12 were given to all patients. After a median follow-up time of 5.5 months (range, 3-8) all patients are alive and evaluable for the response to therapy. According to National Cancer Institute (NCI) criteria 7 patients achieved a PR and 3 CR, whereas 3 were refractory to treatment [2 stable disease (SD), 1 progressive disease (PD)]. Overall 40 courses of R-CEOP have been administered (median 4; range, 2-6). Grade III/IV neutropenia and thrombocytopenia were observed 3 patients. Three episodes of grade III/IV infections were registered. In conclusion, these preliminary results suggest that R + modified-CEOP is an effective and safety approach in patients with advanced B-cell CLL thus representing a possible alternative to the use of R+F or R+FC associations.

PO175
SEQUENTIAL PRESENTATION OF B-CHRONIC LYMPHOCYTIC LEUKEMIA AND CHRONIC MYELOPROLIFERATIVE SYNDROME
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The co-existence of B-chronic lymphocytic leukemia (B-CLL) and chronic myeloproliferative syndrome (CMS) is rare and a limited number of observations are reported in the literature. We report the case of a 69-year-old male who developed CMS after an initial diagnosis of B-CLL. At the initial presentation, two years ago, the patient had leukocytosis (WBC 18.000/mm³) and lymphocytosis (Ly 60%), while the red cells and platelet count were normal. The lymphocyte immunophenotype on peripheral blood was typical of B-CLL (CD5+; CD19+; CD23+; low intensity sIgA). Mild splenomegaly, no lymphadenopathy were found at the clinical examination and confirmed at the total body CT scan. Diagnosis of B-CLL stage II according to Rai classification was made. The patient wasn't treated and he underwent periodi-
cal follow up. About six months later, the hematological screening showed moderate normocytic anemia (Hb 8 g/dL), Coombs ‘test negative, mild increase of white cell count (WBC 20,000/mm³), lymphocytosis (Ly 58%), and severe increase of platelets count (PLT 800,000/mm³) and of LDH (800 U/L). Massive splenomegaly was found at the clinical examination. The bone marrow biopsy pointed out hypercellularity, erytroid and megakaryocytic hyperplasia without fibrosis and showed an interstitial lymphoid infiltration of around 6%. Cytogenetic study on bone marrow showed abnormal but Philadelphia negative, cariotype. No myeloid immature cells were seen in the peripheral blood smears. Lymphocyte immunophenotype on peripheral blood was positive for CD5⁺;CD19⁺; CD23⁺ while BCR/ABL gene rearrangement resulted negative. We conclude that, although rare, the coexistence of B-chronic lymphocytic leukemia and Chronic myeloproliferative syndrome is more often reported in the recent literature a cause of frequent hematologic follow-up. Further cytogenetic and molecular studies are needed to better define the feature and the distinct genomic events which arise these hematological malignances

PO176
A CASE OF B CELL CHRONIC LYMPHOCYTIC LEUKEMIA WITH OSTEOLYTIC BONE LESIONS
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Hypercalcemia and osteolytic bone lesions are common in chronic lymphoproliferative disorders such as adult T-cell leukemia-lymphoma (ATL) associated with infection by Human T-cell leukemia-lymphoma Virus-1 (HTLV-1) or multiple myeloma (MM), but are rarely described in B cell chronic lymphocytic leukemia (B-CLL) (Fain 1994; Lerner 1994). In B-CLL hypercalcemia and osteolytic bone lesions are frequently observed in the context of advanced disease (Marcelli 1988, Briones 1996). Hypercalcemia in patients with ATL is caused by an excessive production by tumor cells of parathyroid hormone-related protein (PTHrP) (Imamura 1992); in B-CLL serum levels of parathyroid hormone (PTH), PTH-related peptide and of several cytokines are normal (Briones 1996). The interleukin-6 (IL-6) is the main growth factor of fresh cells of MM isolated from patients; IL-6 increases in vitro both the formation of osteoclasts and their ability of bone reabsorption; spontaneous production of IL-6 is present in B-CLL, but its role is still unclear. New experimental evidences show that in B-CLL hypercalcemia and osteolytic bone lesions are due to increased bone reabsorption, which might be caused by the secretion of osteoclasm-stimulating factors by the large cell component invading the bone marrow (Beaudreuil 1997). We report the case of a 72 year-old female patient with diagnosis of B-CLL (CD5⁺;CD23⁺; CD25⁺), according to the FAB classification, which developed, during the progression of disease, hypercalcemia and osteolytic bone lesions. The patient was asymptomatic at diagnosis; the serum levels of calcium (8.4 mg/dL), lactate dehydrogenase (LDH) (388 U/L) and of b2 microglobuline (1800 ug/mL) were normal; bone marrow biopsy showed a nodular involvement by malignant B cells: clonal B lymphocytes were mostly of small dimensions with a regular nucleus; the Tomography Computerized Total Body (TC TB) described splenomegaly and lymph nodes only in few sites. The patient, during the progression of disease, was limping by osteolytic bone lesions of the femur; the serum levels of Calcium (14 mg/dL) and b2 microglobuline (3550 ug/mL) were elevated; the serum levels of LDH were normal (332 U/L); according to some studies (Imamura 1992), in our patient, during the progression of disease, the levels of CD25⁺ cells (expression of the IL-2 receptor) in the peripheral blood increased; bone marrow biopsy showed a diffused involvement by malignant B cells: B clonal lymphocytes were of small and large dimensions with an irregular nucleus; the TC TB described lymph nodes with diameter superior to 4 - 5 cm in several sites; the X-ray skeleton described a great number of osteolytic bone lesions mostly of the femur and of the vertebrae. In our patient, during the progression of disease, the serum levels of PTH increased: 90 pg/mL; after chemotherapy of third course with fludarabine (IV cycles overall) the serum levels of PTH decreased: 11 pg/mL. In conclusion hypercalcemia arising in a patient with a low grade lymphoproliferative disorder, as B-CLL, may indicate progression or trasformation of B-CLL in prolymphocytoid leukemia (Lerner 1994) or Richter’s syndrome (Beaudreuil 1997).

PO177
SIMULTANEOUS OCCURRENCE OF CLL AND LMC IN THE SAME PATIENT
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On January 26, 2002, a 60 year-old man, previously healthy, was admitted to our institution after discovery leukocytosis in a routine analysis. There was no previous history o exposure to radiation or myelotoxic drugs. The physical examination was normal. Relevant laboratory were: leucocytes 37.500/µL (a differential count of 44% neutrophils, 48% lymphocytes, 2%
eosinophils, 2% monocytes, 1% basophils, 2% myelocytes, 1% metamyelocytes); platelet count 232,000/µL, hemoglobin was 14.2 grams/desiliter, leucocyte alkaline phosphatase score, lactate dehydrogenase and β-2 microglobulin were normal. A bone marrow aspirate was hypercellular with an increased proportion of myeloid series in all maturative stages; the percentage of lymphocytes was 35%. Bone marrow biopsy showed a marked increase of granulopoietic lineage, with normal maturation, a marked increase of megakaryocytic lineage, a decreased of erythropoiesis, a light reticulin fibrosis and a small lymphoid nodule with mature - appearing lymphocytes. Immune surface marker analysis of blood and bone marrow lymphocytes disclosed 83% of cells to be CD5, CD19, CD23, CD5-CD19 (simultaneous staining) positive, Sig weakly positive, FM C-7 and CD38 negative. The cytogenetic study of the bone marrow revealed a 46, XY, t (9;22) in all metaphases analyzed. The polymerase chain reaction (PCR) analysis showed bcr/abl rearrangement and the rearrangement of the heavy chain immunoglobulin gene (JH). A diagnosis of chronic lymphocytic leukemia (RAI stage 0) with concomittant chronic myeloid leukemia was made. Five weeks later, the patient had a myocardial infarction and was submitted to a rivascularisation procedure. For this reason, the patient couldn't perform the high dose of chemotherapy, but he started the treatment with hydroxyurea. After seventeen months the leucocyte counts maintain between 5 and 20,000/µL. The coexistence of chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) in the same patient has only been reported occasionnally, with most cases corresponding to patient who developed CML during the evolutive course of CLL. In the latter cases, the leukemogenic effect of the treatment employed for CLL and the impaired immune surveillance associated with CLL might be related to the appearance of CML. Some authors were performed the molecular studies attempting to explain the origin of two proliferation cells in the case of simultaneaus occurence of CLL and CML. The coexpression of molecular markers specific of both diseases (bcr/abl rearrangement for CML and the monoclonal pattern of JH for CLL) suggests the different origin of CLL and CML, one myeloid and one lymphoid precursor respectively.
The aim of this study is to define the clinicopathologic features and outcome of non-MALT marginal zone-derived neoplasms: splenic and primary nodal marginal zone lymphoma (MZL). We studied 43 pts, 34 with splenic and 9 with nodal MZL. Biopsy samples from lesional tissues (lymph node or spleen, and/or bone marrow) were available from all patients. The diagnosis was made combining histomorphology, immunophenotype and clinical data. The pattern of nodal involvement varied from sinusal, to nodular/perifollicular, to diffuse; in the spleen the lymphoma usually involved both the white and the red pulp. Lymphoma cells were small to medium in size, had a B-cell immunophenotype and frequent IgM, IgD and bcl-2 protein expression. CD5, CD10, CD25 and bcl-6 were negative in all cases. Of 34 patients with splenic MZL (16 males and 18 females, median age 59 years, range 3-85), 24 (69.4%) presented in stage IV with bone marrow involvement and 10 (29%) peripheral blood involvement. The median hemoglobin level was 12 g/dL and the median platelet count was 126,000/mL. Nine had an M component. Twenty-six patients presented with disease limited to the spleen and bone marrow and locoregional lymph nodes, and 7 (21%) showed disease extension to small superficial and lymph nodes. Seven had liver involvement, 15 had bulky disease and 6 B symptoms. Serology for HCV was positive in 11 of 31 studied patients (35%). Of 9 patients with nodal MZL (4 M/5 F, median age 62, 25-74), 4 (44%) had stage IV disease with bone marrow involvement, 2 had bulky disease, 2 had a poor performance status and in 2 B symptoms were present. Only one case showed blood involvement. HCV serology was positive in 2 patients (22%). The median follow-up for the entire series was 3 years (1-16.4). Of 34 splenic MZL patients, 17 had splenectomy, followed by chemotherapy in 11, 8 had only chemotherapy, 7 were followed without initial treatment. Anti-viral treatment was used in 4 HCV-positive patients: in 2 as first-line therapy, in 1 after a watch-and-wait period (with response of lymphoma in all three), in 1 at progression of HCV infection after initial CHOP with no response. One HCV-positive subject with splenic MZL progressed with extranodal localisations (parotid gland) 5 years after splenectomy. First-line therapy in the 9 nodal cases consisted mainly of CHOP-like chemotherapy. Of splenic MZL patients, 13/27 (48%) obtained a complete remission and 12/27 (44%) a PR. Of nodal MZL patients, 6 (67%) responded to treatment. The median event-free survival (EFS) was 2.8 years for the nodal type and 3.3 for the splenic type (5.1 for cases confined to abdomen and 2.1 for cases with superficial adenopathy). The median overall survival was not reached for either type. Ten patients died. This study indicates that splenic and primary nodal MZL are indolent disorders with high HCV seroprevalence. These two marginal zone-related lymphoma subtypes do, however, have distinct presenting features and show different patterns of dissemination.

PO180

ANTI-CD20 MONOCLONAL ANTIBODY (RITUXIMAB) IN POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: RESPONSE TO TREATMENT AND OUTCOME

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Post-transplant lymphoproliferative disorders (PTLDs) are a severe complication occurring in chronic immunosuppressed transplanted patients (pts). In most cases PTLDs are associated with Epstein-Barr virus infection. The overall mortality rate reported varies from 50 to more than 80% during the first year from diagnosis. The treatment of PTLDs in solid organ transplant recipients is far to be well defined: it varies from reduction of immunosuppressive regimen alone to antiviral and/or chemotherapy. The efficacy of anti-CD20 monoclonal antibody (rituximab) has been proven by several studies, achieving an overall 20-66% response rate. However, its appropriate use has to be better defined, particularly regarding the different histological subgroups of PTLDs (polyclonal-polymorphic vs monoclonal-monomorphic forms), the scheduled treatment (monotherapy vs immuno-chemotherapy combined regimen), and, finally, the risk of relapse at the recovery of EBV-infected B cell compartment. In this study we present the overall response rate and outcome of 13 PTLD pts who received rituximab in our Unit between June 2000 and August 2002. The clinicopathological characteristics of patients are listed in the Table 1. Twelve out of 13 pts received rituximab as first line therapy (2 pts received rituximab alone for four weekly doses, and 10 pts received rituximab plus cyclophosphamide or CHOP). One patient received rituximab plus CHOP as second line therapy. Moreover, in all pts, a reduction of the immunosuppressive regimen was performed during the therapy. Overall, 7/13 (54%) pts entered CR, maintained for a median follow-up of 18 months (range 10-27), while the disease-related mortality rate was 46%. Early relapse (7 months after end of therapy) occurred in one patient. No graft rejection was observed during the treatment. The therapy was well tolerated, and only one patient died because of infection (cytomegalovirus reactivation). In conclusion, our results confirm the efficacy and the feasibility of rituximab therapy, particularly in an immuno-chemotherapy combined regimen, in order to obtain a stable complete remission in aggressive, malignant lymphomas, late PTLDs.
Table 1. Clinico-pathological characteristics of patients.

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<td>13</td>
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PO181
SAFETY AND EFFICACY OF PEGYLATED LIPOSOMAL DOXORUBICIN COMBINED WITH RITUXIMAB AND CHEMOTHERAPY IN FRAIL PATIENTS WITH NON-HODGKIN’S LYMPHOMAS: EXPERIENCE FROM 12 CASES
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CHOP chemotherapy is the standard treatment regimen for patients with intermediate and high grade non-Hodgkin’s lymphoma (NHL), but the complete remission rate is nearly 50%. However, due to the cardiotoxicity of doxorubicin, the use of CHOP is restricted to patients who present a risk for cardiotoxicity. Caelyx is a pegylated liposomal preparation of doxorubicin with no apparent cardiotoxicity at a dose of up to 2 g/m². The outcome for relapsed/refractory NHL patients is limited however not only by the multi-drug resistance and the low performance status but also of the high toxicity of second and third line regimens, causing dose reduction, while in first line NHL patients the age and the important concurrent co-morbidity factors is associated to dose-limiting therapy. We report our experience with CDOP regimen (Cyclophosphamide 750 mg/m² for day 1, pegylated liposomal Doxorubicin 40 mg/m² for day 1, Vincristine 1.4 mg/m² for day 1, Prednisone 100 mg p.o. for 5 days) plus Rituximab (375 mg/m² i.v.) on day 15 of every cycle. The cycle was repeated every 21 days for six cycles. Twelve patients (7 males and 5 females) were treated; median age 69 years (range 57 to 83). Eight patients were refractory or relapsed after different treatment regimens; four patients were in front line-therapy, but with important co-morbidity heart disease. The histologic pattern was:

- diffuse large B cell lymphoma (5),
- B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma (5),
- lymphoplasmocytic lymphoma (1), mantle cell lymphoma (1).

Eight patients (66%) were considered at stage III-IV, according to WHO the performance status was very poor: grade III for 1 patients, grade IV for 3 patients. The treatment was well-tolerated and major toxicities (WHO grade III/IV) did not occur. The administration of liposomal doxorubicin was not accompanied by severe side effects: mild oral mucositis (grade II), neurological toxicity (grade I) were observed in two cases. No evidence of cardiac toxicity and of important hematologic toxicity (G-CSF was used prophylactically) was observed: in fact only one patient delayed therapy for grade II hematologic toxicity. Nine patients completed the planned treatment (six courses), one patient, non responder, stopped therapy after 3 courses. Up to now a complete response was achieved in 4 patients (33%), a partial response in 7 (58%) and one had a stable disease, for an overall response rate of 91%. The median follow-up for all 12 cases was 7 months (range 3 to 10). A reduction of 30% of the dose was effected only in 4 patients. These preliminary results and, first of all, the high tolerability and efficacy of the CDOP/RTX regimen, suggest that the pegylated liposomal doxorubicin may have an important role in the treatment of non-Hodgkin’s lymphoma in frail patients, obtaining an adequate dose intensity without reductions. Funding: Supported in part by AIL Pesaro Onlus.

PO182
ROLE OF CD38 EXPRESSION IN B-CELL NON-HODGKIN’S LYMPHOMAS IN LEUKEMIC PHASE
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Recently, several reports have suggested an association between CD38 expression and worse prognosis in B-cell chronic lymphocytic leukemia (CLL), especially when associated with an unmutated immunoglobulins gene status. The improvement of immunophenotypic techniques and the availability of an increasing number of surface and cytoplasmic monoclonal antibodies allows to recognize a growing proportion of cases which can not be defined as typical CLL. The aim of this study was to evaluate the incidence and prognostic role of CD38 expression in patients with histologically documented B-cell non Hodgkin’s lymphoma (NHL) in leukemic phase. Between January 2001 and December 2002, 78 consecutive, previously untreated patients with NHL in leukemic phase with immunophe-
nototypic features that differed from those of typical CLL (CD5+, sIg+ (weak), CD23−, CD79b+, FMC−7−), were studied. In all patients a specific histologic diagnosis was obtained by lymph node biopsy (18 cases) by bone marrow trephine needle biopsy (12 cases) or both (31 cases).

There were 52 male and 26 females. The median age of the whole population was 58 years, ranging from 36 to 81 years. The clinical and biologic features of the patients enrolled in the study and response to treatment were analyzed and compared according to CD38 expression. A significantly higher proportion of CD38+ cases was found in the more aggressive histotypes with respect to the indolent forms (diffuse large B-cell and mantle cell NHL vs. small lymphocytic, marginal zone, lymphoplasmocytic, follicular and unspecified small cell lymphoma: 75% vs. 44%; p<0.5). A higher proportion of patients with WBC> 20,000, Plts<100,000, increased LDH, more than 5 involved lymph nodes, early need of treatment, was found among CD38+ cases; nevertheless, the difference reached statistical significance only for the number of involved lymph nodes. No relationship between CD38 expression, splenomegaly and presence of bulky disease was found. Furthermore, among those patients for whom a watchful policy was possible, the therapy-free duration was shorter in CD38+ cases. The short follow-up does not allow the assessment of the prognostic importance of CD38 expression on survival. CD38 expression was also evaluated in the normal T-cell population to explore the possibility of a protective effect of an activated T-cell response. Our findings suggest that a higher CD38 expression is more frequently observed in the more aggressive histotypes of NHL in leukemic phase and that this is associated to poor prognostic clinical features. Further studies are needed to fully define the prognostic role of CD38 expression in patients with leukemic NHL.

**PO183**

A BRIEF COURSE OF CHEMO-IMMUNOTHERAPY FND + RITUXIMAB IS EFFECTIVE TO INDUCE A HIGH CLINICAL AND MOLECULAR RESPONSE IN ELDERLY PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMAS AT DIAGNOSIS


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Introduction: Clinical an molecular responses are rarely seen with chemotherapy alone in FL. The addition of Rituximab to fludarabine containing regimens may enhance response and its duration. Patients and Methods: from March 1999 to March 2003, 80 elderly patients (age >60) with advanced stage FL at diagnosis were enrolled into this study. Treatment consisted in: 4 courses of FND (Fludarabine 25 mg/m2 days 1-3, Mitoxantrone 10 mg/m2 day 1 and dexamethasone 20 mg days 1-3) followed by 4 Rituximab infusions at 375 mg/m2/week; pts in partial remission received 2 further FND and 2 Rituximab infusions. PCR molecular monitoring for the presence of IgH/Bcl2 and/or Ig heavy chain gene rearrangement was performed at the beginning of the treatment, after FND, after Rituximab and during follow-up time on bone marrow (BM) samples. Results: median age was 65 (range 60-78); 42 males and 38 females; 13% had stage II, 12% stage III and 75% stage IV disease; 64% had BM involvement; 42% had bulky disease, 28% had 2 extranodal sites and 27% were at high risk according to IIL score. PCR molecular analysis was performed in 54 pts at diagnosis: 63% were BCL2 rearranged and 37% were not. A molecular marker (IgH rearrangement) was detected in 35% of BCL2 negative pts. Up to date, 64 pts are evaluable. Clinical response at the end of the whole treatment program was: CR 72%, CRu 12%, PR 5% and NR 10% and toxic deaths 1%. Complete response (CR+CRu) increased from 44% after FND to 84% with the addition of Rituximab. Seventy per cent of responding pts did so with a very brief chemo-immunotherapy (4 FND + 4 Rituximab). So far a molecular marker of disease was detectable in 28 pts. After FND 7/28 pts did not show anymore BM molecular disease, while PCR negativity was achieved in 22/28 pts after Rituximab treatment. PCR negative status was strongly associated with clinical CR: all PCR negative pts were in CR compared to 66% of PCR+ ones (p<0.05). With a median follow-up of 24 months, 2-yr progression-free-survival was 62% for the whole series of 64 pts. Among the 28 pts who had molecular monitoring, progressions were seen more frequently in PCR+ pts: 3/6 progressed compared to only 2/22 PCR- pts. The whole treatment program was entirely performed in an outpatient setting. The only severe toxicity observed was neutropenia in 22% of FND courses, but only two pts developed bacterial infection and one severe diabetic patient died of neutropenic sepsis during FND chemotherapy. Rituximab toxicity was mild as usually expected. Conclusions. A brief course of chemo-immunotherapy is effective with low toxicity in elderly pts with advanced stage FL. Clinical and molecular responses are improved by the addition of Rituximab to FND. The duration of clinical response is promising and PCR- pts seem to have a lower progression rate.
Post-transplant lymphoproliferative disorders (PTLD) represent a major complication of iatrogenic immunosuppression. Here we aimed at defining the molecular histogenesis of PTLD. Genotypic markers of histogenesis include somatic hypermutation (SHM) of IgV genes, indicating an origin from germinal center (GC) or post-GC B cells. Phenotypic markers of histogenesis are represented by BCL-6, that is restricted to GC B-cells; MUM1, that denotes the final step of intra-GC B-cell differentiation; and CD138, that clusters with post GC B-cell differentiation. The tumor panel was composed by 52 PTLD arising in 51 solid organ transplant recipients and was classified into polymorphic cell lymphoma (PCL; n=12), diffuse large B cell lymphoma (DLBCL; n=36) and Burkitt/Burkitt-like lymphoma (BL/BLL; n=4). Cases were analysed for i) IgV gene usage and mutations; ii) intraclonal heterogeneity of IgV genes; iii) EBV infection; and iv) expression of BCL6, MUM1 and CD138. A functional IgV rearrangement was obtained from 33/41 (80.5%) PTLD. In 8/41 (19.5%) PTLD, the only IgV rearrangement was nonfunctional because of crippling mutations (5 cases) or because of an out-of-frame rearrangement (3 cases). SHM of IgVH genes was detected in 29/33 PTLD (87.9%) showing functional IgVH rearrangements and in 6/8 (75.0%) PTLD showing nonfunctional IgVH rearrangements. The distribution of replacement (R) and silent (S) mutations was analysed by the binomial and the multinomial distribution models. A lower than expected number of R mutations in the FR, suggesting antigen stimulation, was detected in 12/29 (41.4%) PTLD, while a higher than expected number of R mutations in the CDR, suggesting antigen selection, was observed in 9/29 (31.0%) cases. Intraclonal heterogeneity of IgV genes, reflecting ongoing SHM, was detected in a fraction of BL/BLL and DLBCL centroblastic. Expression analysis of BCL6, MUM1 and CD138 showed that BL/BLL and DLBCL centroblastic preferentially associate with the BCL6+/MUM1-/CD138− profile or the BCL6+/MUM1+/CD138+ pattern typical of post-GC B cells. EBV infection was detected in 30/52 (57.7%) PTLD, without significant differences among histologic subtypes. The implications of these data are manifold. First, most PTLD derive from GC-experienced B cells. Second, despite a common derivation from GC-experienced B cells, the precise histogenesis of single PTLD cases displays a certain degree of molecular and phenotypic heterogeneity, leading to the recognition of biologically homogeneous PTLD categories. Third, a subset of PTLD harbor crippling mutations of IgVH and/or IgVL genes. Because a functional B cell receptor is required for survival of normal B cells during GC transit and may be necessary also for many lymphomas, it is conceivable that PTLD cells are rescued from apoptosis through mechanisms independent of antigen priming. Fourth, a small group of PTLD display germline IgVH genes and may derive from truly pre-GC B cells or, alternatively, from B cells that have transited through the GC but have been impaired in exerting a full GC-reaction.
surviving with a median observation time of 31 months (range 10-60). Thirty-five patients are still in CR. The 4-year probability of overall survival and failure-free survival are 78.2% and 45%, respectively. Sixty per cent of patients experienced grade III-IV granulocytopenia. Two patients suffered grade III pulmonary infection and one grade III liver toxicity. Grade III-IV anemia and thrombocytopenia was observed in 22% of patients. In a subset of 46 patients, bcl-2 translocation was positive in 36 patients. At the end of treatment, 25 of these patients had CR and 19 (76%) converted to polymerase chain reaction (PCR) negativity. Flu/CY/MITO regimen has a high level of activity in follicular lymphoma. Toxicity is acceptable and the treatment is made feasible by the use of antibiotic prophylaxis with G-CSF. The conversion of bcl-2 from positive to negative by polymerase chain reaction (PCR) in bone marrow and/or peripheral blood suggests a possible role for this treatment in clearing minimal residual disease and improving patients' outcome.

PO186
VEBEP AND LOW-DOSE RADIOTHERAPY: A STAGE-TAILORED, VINOERELINE-CONTAINING APPROACH FOR NEWLY DIAGNOSED HODGKIN'S DISEASE
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Background: Following the encouraging results of our previous VEBEP and of single agent vinorelbine (VNR) in pretreated HD, a new VEBEP regimen was developed at our institutions with the primary aim to reduce short and long-term toxicity and, if possible, to improve therapeutic outcome. Patients and methods: The regimen consisted in epirubicin 30 mg/m^2 iv day 1-3, cyclophosphamide 1000 mg/m^2 iv on day 1, VNR 25 mg/m^2 iv on day 2, bleomycin 10 mg/m^2 iv on day 3, and prednisone 100 mg iv day 1-3. Courses were given outpatient every 21 days without growth factor support, unless requested according to ASCO guidelines. Treatment plan varied on the basis of Ann Arbor/Cotswold stage: early stages (I-III A +/-E) were given two courses followed by involved-field radiotherapy (IF RT) 25-30 Gy (Group 1: G1), intermediate stages (IIA with mediastinal involvement, I-III B/IIA/B bulky +/- E) were given four courses of VEBEP and IF RT at same doses (Group 2: G2), and advanced stages (all others, Group 3 or G3) were given six courses of VEBEP with RT only on bulky sites. RT was delivered only if complete remission (CR) was confirmed by restaging procedure at the end of the chemotherapeutic program. Results: from 11/97 to 05/03, 134 consecutive patients have been accrued. Of the 112 evaluable (treatment concluded) main clinical characteristics were: M:F 57:55, median age 36 years (range 15-78); G1/G2/G3: 35/38/39 patients, respectively. After treatment, 35/35 (100%), 36/38 (95%), and 31/39 (79%) patients of each group were in complete remission/complete remission-unconfirmed. Toxicity was globally mild. No patient was hospitalized for management of toxic effect, and only one received RBC transfusion. Eleven percent of courses were given at reduced dose or were delayed due to incomplete hematologic recovery, and GCSF was given to three patients. Fever, mucositis and peripheral neurotoxicity never exceeded grade II. After a median follow-up of 21 months (range 1-61), eighteen patients with relapse (N=8) or resistant disease (N=10, 2 from G2 and 8 from G3, defined as non-compete remission) underwent 2nd line treatment. Thus, actuarial 2-year freedom from progression and overall survival, are 96, 84, 78% and 100, 90, 88% for the three prognostic groups, respectively. Conclusions: Stage-modulated VEBEP chemotherapy and low-dose RT is highly effective in HD and carries a low-acute toxicity profile. Longer follow-up is needed to confirm therapeutic results and to evaluate long-term sequelae. An increase in dose-intensity could be planned for patients at higher risk.

PO187
DOSE-ADJUSTED ABVD PLUS G-CSF CAN MAINTAIN HIGH RELATIVE DOSE INTENSITY AND MAY IMPROVE COMPLETE RESPONSE AND SURVIVAL RATES IN PATIENTS WITH ADVANCED HODGKIN'S LYMPHOMA
Russo F, Celentano E, Corazzelli G, Frigeri F, Maracci G, Swanera G, Pinto A
unità di Ematologia Oncologica Istituto Nazionale Tumori Napoli, Italy

Given its favourable toxicity profile 6 to 8 cycles of ABVD (±small field radiotherapy to residual sites) are currently regarded as the standard treatment in advanced Hodgkin's lymphoma. This treatment produces complete response (CR) and freedom from progression (FFP) rates of 80% and 65% respectively. Novel, intense combined regimens (Stanford V, BEACOPP) may reduce treatment failure but do not significantly improve overall survival. In addition, the more intensive regimens generally have more negative toxic events. The goal of primary treatment is to maximize the cure of Hodgkin's Lymphoma (HL) with the minimum of cardiac toxicity and long term effects and it may be possible to reach this objective by improving the performance of ABVD. Retrospective studies on our series of patients with advanced Hodgkin's Lymphoma undergoing their first chemotherapy treatment, revealed that
the most common factor in treatment failure was a suboptimal drug dose intensity, mainly due to delay in therapy and decreasing drug doses induced by persistence of myelosuppression at recycling. The literature on this topic generally omits details of dose intensity or reports actual doses lower than those planned by the protocols. To improve ABVD results we developed a protocol which adds G-CSF (5 µg/kg/daily from d8 to d11) to the standard ABVD treatment and uses more stringent criteria for dose reduction or delay in treatment together with dose compensation so as to maintain a relative dose intensity RDI near 1 (according to Hryniuk). From March 1997 to April 2002 33 patients with advanced HD were treated with this new protocol and 23 with a standard ABVD protocol. The results were also compared with a historical group of 56 patients who had undergone a hybrid MOPP/ABVD treatment. In Table 1 we report the outcome of this 3 subsets of advanced HD patients CR rates of groups 1, 2 and 3 were 100%, 91%, and 73%, respectively FFP and OS rates were significantly higher in the 33 patients of the ABVD + G-CSF subset (FFP 97% vs 64%, p=0.027: OS 97% vs 76%; p=0.039). Patients who received G-CSF showed the best toxicity profile and were all able to maintain a RDI near 1 without any delay in recycling. Patients treated with standard ABVD had good toxicity profiles but the RDIs were about 20% lower than the planned dose intensity. Hybrid MOPP/ABVD had significantly acute toxicity compared to ABVD treatments and showed the lowest RDIs (25% off the planned DI). We believe that this improved outcome in our G-CSF patients is due to the increased RDI, though a synergistic action of G-CSF and ABVD cannot be excluded.

<table>
<thead>
<tr>
<th>Group</th>
<th>N°</th>
<th>IPS</th>
<th>CR ≥ 2 vs 0-1</th>
<th>FFP</th>
<th>OS</th>
<th>OS RDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ABVD + G-CSF</td>
<td>33</td>
<td>20 13</td>
<td>100% 97%</td>
<td>p=0.003 3%</td>
<td>p=0.0039</td>
<td>1.01 (0.94-1.12)</td>
</tr>
<tr>
<td>2. stand ABVD</td>
<td>23</td>
<td>14 9</td>
<td>91% 64%</td>
<td>p=0.027</td>
<td>OS 97% vs 76%</td>
<td>p=0.039</td>
</tr>
<tr>
<td>3. MOPP/ABVD</td>
<td>56</td>
<td>34 22</td>
<td>73% 68%</td>
<td>p=0.003</td>
<td>OS 97% vs 76%</td>
<td>p=0.039</td>
</tr>
</tbody>
</table>

IPS= international prognostic score; RDI = relative dose-intensity; FFP= freedom from progression; OS= overall survival.

PO188
TREATMENT OF AGGRESSIVE B NON-HODGKIN'S LYMPHOMA WITH CHEMOTHERAPY AND RITUXIMAB DURING PREGNANCY
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The occurrence of aggressive B non-Hodgkin's lymphoma during pregnancy rises an ethical dilemma: intensive chemotherapy is mandatory to give the mother a reasonable chance of cure, but it has potential risk of fetal injury. Recently efficacy and safety of Rituximab combined with non-alkylating chemotherapy during pregnancy was firstly reported. We report a case of a 34-year-old female affected by stage II A bulky diffuse large B cell lymphoma (CD20+) diagnosed at the 22 week of gestation, who rapidly developed a superior vena cava syndrome (SVCS). After having obtained written informed consent, at the 25th week of gestation we started treatment, with Rituximab 375 mg/m² day 1, Doxorubicin 50mg/m² day 1, Vincristine 2 mg (total dose) day 1, Prednisone 100 mg (total dose) day 1 to day 5 given. The cycles were given at 2 weeks cycle intervals. The symptoms related to SVCS rapidly improved. Fifteen days after the third cycle of chemotherapy the patient developed bilateral pneumonia that required assistance in intensive care unit and assisted ventilation. For this problem at 32 weeks of gestation the patient underwent caesarian section and delivered a healthy male. Clinical and radiological findings improved in 10 days after large spectrum antifective therapy. No microbiological agents were find in the specimens. Three weeks after delivery, three more cycles of chemotherapy were given, with Rituximab 375 mg/m² day 1, Doxorubicin 50mg/m² day 1, Vincristine 2 mg (total dose) day 1, Cyclophosphamide 750 mg/m², Prednisone 100 mg (total dose) day 1 to day 5 given in 3 week cycle. Involved field radiotherapy as consolidation (30 Gy) completed the treatment. The restaging showed a complete remission. The baby developed well, without infectious problems: at birth and at 40 days of life (CD19/20+) B cells were no detectable in the peripheral blood, but normal peripheral B-cell population (22% CD19/20+; 1300/µL) was observed from the sixth month. B-cell (CD19/20+) became undetectable in the peripheral blood of the patient from the 4th week after the first dose of Rituximab to 12 months later. Serum levels of immunoglobulin were normal. At present, 20 months after the beginning of the treatment, the CD19/20+ cells are 236/µL and the patient is well, in stable continuous complete remission. Our data confirm that Rituximab cross the placental barrier and affect the fetal B-cell subset, but in our case it was safe and recovery was noted after few months. In our case the administration of Rituximab with chemotherapy confirm their efficacy and safety for fetus in pregnancy, as reported by Herold et al. The evaluation of adjunctive infectious risk remains a problem to be analyzed in a larger series of patients.

PO189
Not published
Metabolizing Enzymes in Non-Hodgkin’s Lymphoma: Role of Genetic Polymorphisms

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Everyone has a unique combination of polymorphic traits that modify susceptibility and response to drugs, chemicals and carcinogenic exposures. Toxicants to which an individual is exposed are biotransformed and eliminated from the body after metabolic conversion mediated by Phase I and Phase II xenobiotic-metabolizing enzymes. Phase I enzymes catalyze hydroxylation, reduction and oxidation reactions of xenobiotics (carcinogens/drugs), often converting them into more active or toxic compounds. Phase II enzymes catalyze conjugation reactions (glucuronidation, acetylation, methylation), thereby converting the metabolites into non-reactive, water-soluble products that are eliminated from the organism. Genetic polymorphism in the genes encoding for drug-metabolizing enzymes, underlying the variation in enzyme activity, can modify individual susceptibility to cancers as well as the response to therapy. We studied different common polymorphisms in the genes encoding for cytochromes CYP3A4 and CYP2E1, glutathione S-transferases (GST-M1), NAD(P):quinone oxidoreductase (NQO1) and N-acetyltransferase (NAT1,NAT2) in 47 patients affected by non-Hodgkin’s lymphoma (NHL). A total of 21 allelic variants were evaluated, in particular the null genotype for GST-M1, three CYP3A4 single nucleotide polymorphisms (SNPs): A-392G, T15615C and T20072C, two CYP2E1 SNP: C1053T and G1293C, SNP C609T for NQO1 and a total of 14 SNPs variously represented within the NAT1 and NAT2 genes categories known as slow or fast acetylators. Sequences and selected polymorphisms were retrieved from www.ncbi.nlm.gov/ website. Genotypes were examined by a polymerase chain reaction (PCR) approach. Briefly, sequences of interest including the polymorphic sites were amplified by a first PCR followed by a second single base extension reaction using fluorescently labelled oligonucleotides appropriately designed to detect the SNPs. The latter reaction was performed using the SNAPSHOT assay (Applied Biosystem). SNP-PCR products were detected by capillary electrophoresis on a DNA Genetic Analyzer (ABI PRISM 3100 Applied Biosystem, CA). Collected data were visualized on a fluorescent histogram and analyzed by using ABI GeneScan software (Applied Biosystem, CA, USA). The analyzed NHL patients showed an incidence of CYP3A4, CYP2E1, NAT 1 and NAT2 genotypes similar to that reported in the literature for control groups. In fact we found only one heterozygosity for CYP3A4 (2.2%) out of 43 valuable cases; one patient out of 45 carried the CYP2E1*S allele (2.2%); 20 cases of 36 (54.6%) showed a NAT2 slow acetylator genotype and 4 out of 44 (10.3%) had a NAT1 slow genotype. As far as the NQO1 genotype, we report a slightly higher prevalence of the C609T variant among NHL patients. We found 17 out of 47 cases carrying the C609T allele (36.2%) whereas a 13-25% frequency in control subjects was reported by comparable studies in Caucasian, in Europe and North America. Furthermore the GST-M1 null frequency we found is consistently lower (18%) compared to what reported in controls (41-54%). Albeit preliminary, our data show similar conclusions for some respect to those reached in previous investigations while identifying a different percentage of NQO1 609T genotype among tumors. A number of studies have investigated drug metabolizing enzymes polymorphisms but their analysis was restricted to evaluate single or a limited number of SNPs. In our opinion the best approach is to extend the analysis to many candidates in order to represent a most comprehensive allelotype profile allowing for further, more refined associations between xenobiotic metabolizing pathways, the risk of cancer and response to therapy. We realize more patients need to be genotyped to support solid conclusions moreover we need to perform a case control study to correctly evaluate the allelic frequencies in our population.

Lymphocyte Predominance Hodgkin Disease. Results from Single Centre Experience

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Recently significant progresses has been done on the biology of Hodgkin Disease; however few information are available on the role of chemotherapoy in lymphocyte predominance variant of Hodgkin lymphoma in literature. Because large prospective multi-center randomized studies are missing, information on survival probability can be drawn from uni-center retrospective analyses. With this purpose we analyzed our >20 years experience in Lymphocyte predominance Hodgkin Disease. From January 1975 to May 2003 sixty-five consecutive patients with lymphocyte predominance Hodgkin’s disease were diagnosed at Hematology Unit of Businco Hospital in Cagliari. Forty-four were male and 21 female, mean age was 34 years ranging from 14 to 75 years. Sixteen patients presented with Ann Arbor stage I, 31 with stage II, 12 with stage III and 6 with stage IV. Five patients had a bulky mass, one additional patient had an extranodal disease and 14 had severe splenomegaly at presentation. Patients were treated with the following modalities according to stage and
protocol in use: 36 patients received chemotherapy, 14 radiotherapy and 15 combination of both. During the observation period 11 patients died: seven by progressive disease or by therapy related causes. Four patients died while in complete remission for disease not related causes. Overall eighteen patients relapsed or had refractory disease. After a median follow up of 112 months (range 10-300), overall survival and disease free survival were 75% (95% confidence interval, 51%-87%) and 46% (95% confidence interval, 29%-67%), respectively. Both curves plateaued after 160 months of follow up. This retrospective single centre study underline the good prognosis of lymphocyte predominance Hodgkin Disease. Large multi-centre study are necessary to confirm this data in a larger cohort of patients.

PO192
FDG-PET ROLE IN STAGING, THERAPY RESPONSE EVALUATION AND FOLLOW UP OF PATIENTS WITH HODGKIN'S DISEASE: A SINGLE CENTRE EXPERIENCE

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Aim: The purpose of this study is to evaluate the role of FDG-PET in the management of patients (pts) with Hodgkin's disease (HD). Material and Methods: We evaluated 63 consecutive pts with HD who underwent whole-body FDG-PET (108 studies) from June 2000 to February 2003 in our PET centre. PET scans were performed for staging (group a:11 studies), early response evaluation during chemotherapy (group b: 7 studies), restaging at the end of therapy (group c: 22 studies) and follow up (group d: 68 studies). All pts were studied according to the recommended guidelines. In 91 studies both CT and FDG-PET were performed within 4 weeks. Results: Group a - CT and PET showed the same results in 7 pts, while PET revealed more sites of disease than CT in 4 (38%), changing stage and treatment in 2 (18%) Group b - 3 cases had PET alone (2 negatives and 1 positives); in 4 pts with PET and CT studies there was PET/CT correspondence in 1 (persistence of disease), CT positive and PET negative in 3 (at the end of treatment all 3 pts were in complete remission CR). Group c - 8 pts had negative CT and PET (follow up confirmed CR); 3 pts had positive CT and PET in 1 case in different sites; biopsy confirmed PET results in 2 and CT sites in 1; 10 pts had negative PET and positive CT (follow up confirmed CR); 1 pt had positive PET and negative CT (not valuable for insufficient follow up). Sensitivity and specificity were respectively 100% and 95% for PET and 50% and 44% for CT. Group d - 14 cases had PET alone: 11 negatives (confirmed CR) and 3 positives (biopsy and/or follow up not confirmed disease); 54 cases had PET and CT: 31 were PET/CT negative (confirmed persistent CR); 6 cases were PET negative/CT positive (confirmed persistent CR); 12 cases were PET/CT positive (8 relapses, 4 CR); 5 were PET positive/CT negative (1 relapse, 4 CR). Sensitivity and specificity were respectively 100% and 81% for PET and 88% and 77% for CT. Conclusions: In staging PET allows a better diagnostic definition resulting in stage and treatment modification in 18% of patients. During chemotherapy PET seems be an early index of patient outcome but this is only a preliminary result and need a confirm with higher number of cases. PET is superior to CT highly in the evaluation at the end of therapy and lightly also in follow up.

PO193
THE GISL EXPERIENCE WITH VBM CHEMOTHERAPY FOR EARLY-STAGE HODGKIN'S LYMPHOMA PATIENTS

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Aim: The acknowledged effectiveness of VBM chemotherapy (vinblastine, bleomycin and methotrexate) in early-stage Hodgkin's lymphoma has been associated with conflicting toxicity reports. One hundred forty-three patients had been clinically evaluated as favorable stage IA or IIA. Ninety-three patients were treated with the standard VBM schedule combined with extended-field radiotherapy (EF-RT) leaving the choice of the therapeutic sequence free. Fifty subsequent patients were treated with a slightly modified VBM schedule (VbMp) combined with radiotherapy limited to involved fields (IF-RT) and delivered only after the end of chemotherapy. In the VbMp schedule intervals between cycles were 21 days instead of 28, bleomycin doses were reduced, small doses of prednisone were given orally and the interval before radiotherapy was prolonged. Clinical response was complete in 96% of the patients treated with VBM + EF-RT and in 94% of those with VbMp + IF-RT. Relapse rates were nearly identical (12 and 11%, respectively) though over a necessarily different follow-up (91 and 33 months, respectively). Hematological toxicity was tolerable in both trials and pulmonary side-effects were moderate in the first tri-
al and negligible in the second. On the whole, treatment was better tolerated when radiotherapy followed chemotherapy. The VBM regimen confirms to be effective in early-stage Hodgkin’s lymphoma. Administering all cycles before radiotherapy improves tolerance; pulmonary toxicity is mitigated by reduced bleomycin dose, mild prednisone therapy and a more prolonged resting interval before radiotherapy. A slightly higher relapse rate is expectable in the VbM+IF-RT trial versus the VBM regimen if radiotherapy was administered before chemotherapy. The VBM regimen confirms to be effective in early-stage Hodgkin’s lymphoma. Administering all cycles before radiotherapy improves tolerance; pulmonary toxicity is mitigated by reduced bleomycin dose, mild prednisone therapy and a more prolonged resting interval before radiotherapy. A slightly higher relapse rate is expectable in the VbM+IF-RT trial versus the VBM regimen if radiotherapy was administered before chemotherapy. The VBM regimen confirms to be effective in early-stage Hodgkin’s lymphoma. Administering all cycles before radiotherapy improves tolerance; pulmonary toxicity is mitigated by reduced bleomycin dose, mild prednisone therapy and a more prolonged resting interval before radiotherapy. A slightly higher relapse rate is expectable in the VbM+IF-RT trial versus the VBM regimen if radiotherapy was administered before chemotherapy.

In this retrospective study we examine the patient characteristics and outcome of patients with aggressive NHL treated with HDT and autologous transplantation at our Institute from 1982 to 1999. A retrospective analysis was performed examining patient characteristics, prior chemotherapy regimens, pretransplant disease status, HDT regimen, source of stem cells, time for hematopoietic recovery, complications of transplantation, response rates, overall survival (OS) and relapse-free survival (RFS). One hundred thirty-four patients with aggressive NHL were treated with estimated 10-year OS and RFS rates of 50% and 66%, respectively. Disease status (sensitive vs. refractory) pre-HDT was the most powerful predictive parameter for OS and RFS, at both univariate and multivariate analysis. For the entire cohort, transplant-related mortality was only 3.5% without evidence of second malignancies. Our results confirm that HDT with autologous transplantation is associated with a durable RFS in a remarkable proportion of aggressive NHL patients with very low global early and late toxicity. Improved patient selection, transplant timing, ongoing improvements in supportive care, and selected phase III trials should increase outcomes further.

In the Institute we performed a sequential chemotherapy treatment for the indolent non-Hodgkin’s lymphoma (NHL) patients with high-risk presentation at diagnosis. The inclusion criteria were: age between 18 and 55 years; stage II-IV; CD20 positive; an IPI score < 2 or an IPI score =1 with an other poor prognostic factor such as bulky disease or B symptoms. The schedule of this sequential treatment was represented by: phase I, conventional CHOP for 4 cycles; phase II, high dose cyclophosphamide (7 g/m^2) plus G-CSF with peripheral blood stem cells (PBSC) mobilization (with Rituximab on day +3 and +11); phase III, conventional Fludarabine, cyclophosphamide and Rituximab regimen for 4 courses; phase IV, BEAM conditioning regimen with (PBSC) reinfusion. At this time 13 patients were enrolled. Patients’ characteristics included median age of 40.7 years (37-56); 8 male, 5 female; 10 pts were in stage IV and 3 in stage II. According to histology there were 12 follicular lymphoma, and 1 CLL-like lymphoma. Once the therapy program was terminated, all patients underwent clinical restaging (in addition, for the follicular bcl-2 positive patients there was a molecular restaging evaluating bcl-2 on peripheral blood and bone marrow). At the end of treatment all the nine evaluable patients obtained CR. Concerning the bcl-2 follicular lymphoma patients, all obtained a clinical CR and molecular response. Mobilization of PBSC was successful in all the patients with only one leukapheresis. All the patients have completed all the phases of the sequential treatment without significant delays due to toxicity of any kind. Our preliminary data on this sequential modality seem to be very promising but we need more patients and follow-up.

Follicular lymphoma is characterized in 80-90% of patients by the presence of t(14;18)(q32;q21) which involves bcl2 gene. Breakpoints on bcl2 gene may occur at the major or minor cluster regions (MBCR or mcr). The mechanism of action of Bcl2 is not yet clarified. Some studies have demonstrated that bcl2 is involved in the regulation of cellular apoptosis, through modification of mitochondrial membrane potential and/or Ca2+ ions.
flux across the endoplasmic reticulum. Bcl2 rearrangement analysis is an useful tool to perform an early evaluation of the efficacy of intensive chemotherapy or transplants. Our study was done on seven patients with low-grade non Hodgkin’s lymphoma (LG-NHL) who reached clinical and immunophenotypical remission after fludarabine/mytobantrone/cyclophosphamide regimen. We utilized both the intrinsic Bcl2 marker and the extrinsic JH clonal rearrangement in order to assess the minimal residual disease status. The molecular analysis, done on lymphonode samples at diagnosis, was aimed at identifying JH/Bcl-2 and clonal JH rearrangements. The same study was subsequently performed in peripheral blood cells and in bone marrow (BM) samples, both at diagnosis and after 3-6 cycles of chemotherapy. These 7 patients at diagnosis were positive for Bcl2 rearrangement. The BCL-2 MBR was observed in 5 patients and the mcr in 2. In all the patients the analysis performed on lymph-node and marrow samples detected the same Bcl2 and JH rearrangements. In 3 patients the molecular study was done also on peripheral blood mononuclear cells, and detected the same clonal markers. In six out of the seven patients studied after the achievement of CR JH/Bcl-2 rearrangement was undetectable. All patients still showed monoclonal JH rearrangement. One patient, without histological evidence of bone marrow lymphoid infiltration at diagnosis, but with positive molecular analysis on marrow cells, resulted negative for both markers during controls after chemotherapy. Our study, through the use of both intrinsic and extrinsic clonal markers, may shed some light on the multistep pathogenesis of low grade lymphoid neoplasm. One might in fact speculate that the Bcl-2 rearrangement is a late event, occurring in an already transformed cell bearing clonal JH rearrangement. Further studies are needed to assess the clinical value of monitoring those clonal markers, both after standard and high dose therapy.

PO197
IFOSFAMIDE, EPIRUBICIN, ETOPOSIDE AND AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL TRANSPLANT: A FEASIBLE AND EFFECTIVE SALVAGE TREATMENT FOR REFRACTORY AND/OR RELAPSED LYMPHOID MALIGNANCIES
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Background and aims. Primary end points of the study were to confirm the feasibility and the efficacy of the combination of ifosfamide, etoposide, and epirubicin in relapsed and/or refractory lymphoid malignancies and in particular the efficiency of this regimen in mobi-

lizing peripheral blood stem cells. Patients and methods. The combination of ifosfamide (2.5 g/m²), etoposide (150 mg/m²) on days 1-3, and epirubicin (100 mg/m²) on day 1 (IEV) was administered to patients with refractory and or relapsed lymphoid malignancies as part of high-dose program including autologous peripheral blood progenitor cell transplantation (ASCT). Fifty-three consecutive patients with Hodgkin’s lymphoma (HD, 14), aggressive non Hodgkin’s lymphoma (NHL, 19), low-grade non Hodgkin’s lymphoma (LG-NHL, 6), acute lymphoblastic leukemia (ALL, 5), multiple myeloma (MM, 7), other lymphoma (2) entered the study between January 2000 and March 2003. Patients had received a median of two regimens (range 1-10). Median age was 53 years (range 20-80); male were 26, females 27. Among lymphomas (41) the clinical stage was II in 8 patients, III in 8 and IV in 25; 8 symptoms were detected in 13 patients. LDH serum level were above the normal range in 25 patients (47%). Results. Patients were given a median of 2 courses of IEV (1 course in 19, 2 courses in 16, 3 in 16, 4 in 2 patients). They experienced nausea and vomiting (24, 45%), diarrhea (8, 15%), mucositis (6, 11%), oral candidiasis (3, <1%) but no severe infections. The median times to PMN recovery (>0.5×10⁹/L) and platelet recovery (>50×10⁹/L) were 10 days (range 1-25) and 11 days (1-38), respectively. Twenty-one patients received a median of 2 packed RBC units (1-7) and 17 were transfused with a median of 2 platelet units (1-10). A median of 2 apheresis (1-4) allowed the collection of a median of 11×10⁹/L CD34+ cells / kg (6, 8-29) in all responsive patients, after administration of G-CSF (a median of 8 days). In 72% of responsive patients the collected dose of CD34+ cells was sufficient to perform HDT. The outcome is reported in the following Table.

Table. Outcome of patients treated with IEV.

<table>
<thead>
<tr>
<th>CR (%)</th>
<th>PR (%)</th>
<th>HDT</th>
<th>Rel.</th>
<th>DFS mm</th>
<th>Surv mm</th>
<th>A/D</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD (14)</td>
<td>11(79)</td>
<td>2(14)</td>
<td>11</td>
<td>1</td>
<td>165(41)</td>
<td>175(42)</td>
</tr>
<tr>
<td>LC-NHL (16)</td>
<td>7(43)</td>
<td>4(25)</td>
<td>8</td>
<td>—</td>
<td>204(27)</td>
<td>141(28)</td>
</tr>
<tr>
<td>MC-NHL (3)</td>
<td>1(33)</td>
<td>—</td>
<td>2</td>
<td>—</td>
<td>nd</td>
<td>7 (6-8)</td>
</tr>
<tr>
<td>LG-NHL (6)</td>
<td>—</td>
<td>1(16)</td>
<td>2</td>
<td>—</td>
<td>nd</td>
<td>4 (1-25)</td>
</tr>
<tr>
<td>MM (7)</td>
<td>1(14)</td>
<td>3(43)</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>112(17)</td>
</tr>
<tr>
<td>ALL (5)</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>4 (1-13)</td>
</tr>
</tbody>
</table>

haematologica vol. 88[suppl. n. 15]:october 2003
High dose therapy (HDT) with reinfusion of autologous peripheral blood progenitor cells was performed in 26 patients (49%), with allogeneic stem cells in 3 patients (5%). Conclusions. In conclusion IVF proved to be a feasible and effective salvage regimen in refractory HD and in diffuse LCNHL. In addition it efficiently mobilized peripheral progenitor cells in pre-treated patients with lymphoid malignancies with little or no side effects.

PO198
TREATMENT OF LARGE CELL LYMPHOMA IN ADULT PATIENTS WITH BAVEC-MIMA REGIMEN: TEN YEAR EXPERIENCE
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Background: To improve the response rate for aggressive non-Hodgkin's lymphoma (NHL), different chemotherapy regimens have been developed over the past 20 years. None has been proved to be clearly superior to CHOP regimen in randomized studies. Patients and methods: Starting from 1989 we have prospectively used a third generation regimen named BAVEC-MIMA for the treatment of adult patients with high grade NHL. We treated from August 1989 to March 1999 101 patients with median age of 41 years (18-64). Inclusion criteria were: age less than 65 years, all stages, histologic subgroups G and H according to the Working Formulation and diffuse large B-cell lymphoma according to the REAL classification, no previous therapy and performance status less than three. The schedule of this regimen was: BCNU 100 mg/m² day 1; adriamycin 50 mg/m² day 1; etoposide 60 mg/m² days 1-4; vincristine 1 mg/m² day 2; cyclophosphamide 600 mg/m² days 3, 4; aracytin 300 mg/m², 4 hours continuous infusion, day 18; mitoxantrone 10 mg/m² day 19; methotrexate 150 mg/m² day 20; citrovorum factor 15 mg/m² orally day 21; prednisone 40 mg/m² orally from day 1 to 21. Cotrimoxazole was administered from day 9 to day 27. Cycles were repeated every 21 days starting from the end of the previous cycle. Scheduling is characterized by two phases with the aim of killing residual cells by administering non cross-resistant drugs after the intervel. The characteristics of patients were the following: bone marrow involvement 10%, B symptoms 39%, performance status (WHO) 0 = 61%, 1 = 25%, 2 = 14%. According to IPI, which was evaluable in 96 patients, low risk patients were 25%, low-intermediate 47%, high-intermediate 20% and high risk 8%. All patients were treated as outpatients. Results: Seventy-five patients (72%) showed an increased LDH value. Six patients were stage II, 3 stage III and 9 stage IV. Six /18 (33%) patients presented B symptoms, 4 bone marrow involvement and 13/18 (72%) patients showed an increased LDH value. According to IPI score 1 patient was low risk, 9 low-
apy achieved a good partial remission in the six cycles of NHL in IV stage A non responder at the first line therapy. The second case a 67-year-old female with follicular lymphoma achieved a complete remission after four cycles; now, after 12 months, she is still in remission: a partial remission occurred for a concomitant breast cancer. The median age was 79 years (range 67 to 83). The first cycle was very well tolerated and we did not observe any important hematologic and extrahematological toxicity. In conclusion, the low toxicity profile suggests that pegylated liposomal doxorubicin is effective in frail patients and, in particular, a two-weekly dose is well tolerated in old patients.

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PO200
LIPOSOMAL PEGYLATED DOXORUBICIN AS A SINGLE AGENT IN NON-HODGKIN’S LYMPHOMAS OF THE ELDERLY: A SINGLE CENTER EXPERIENCE
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PO201
AGGRESSIVE NATURAL KILLER CELL LEUKAEMIA/LYMPHOMA: DISTINCTIVE CLINICAL AND BIOLOGICAL FEATURES OF ONE CASE
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Natural killer (NK) cells are large granular lymphocytes with innate immune function, playing an important role in the early host defence against viral, bacterial and other infectious agents and against cancer. NK cell tumours are extremely rare diseases accounting for a minority of leukemias and lymphomas. The most common type is the extranodal NK/T cell, nasal and nasal-type lymphoma, which has a typical angiocentric and angiodestructive growth pattern. Aggressive NK leukemia/lymphoma is much more uncommon and few cases have been reported. A 76-year old white woman presented in April 2003 to the Emergency Department of Ospedale Maggiore of Milan, complaining fatigue, dyspnea and fever. Her past medical history was characterised by HCV-related cirrhosis. Physical examination revealed massive enlargement of liver, spleen and lymph nodes. Laboratory examination showed an elevated white blood cell count (WBC 162×10^9/L) and thrombocytopenia (PLT 89×10^9/L); peripheral blood smear revealed large undifferentiated blasts with slightly granular abundant cytoplasm and multinucleated nuclei. No autoantibodies were found; Ig levels were normal except for IgG increase and a slight monoclonal IgGk was detected. Multicolour flow cytometry showed the pathologic cells to be positive for CD1a, CD2, CD4, CD16, CD43, CD30, CD56, CD11c and CD38; furthermore cyCD3, CD33 and CD13 were negative. These data were conceivable with the diagnosis of aggressive NK leukemia/lymphoma. Furthermore a hypertriploid karyotype was detected in most of the metaphases exam-
PO202

CLINICAL AND PROGNOSTIC FEATURES OF INDOLENT B-CELL NONFOLLICULAR LYMPHOMA: A RETROSPECTIVE STUDY ON 373 PATIENTS PERFORMED BY INTERGRUPPO ITALIANO LINFOMI

Vitolo U,1 Luminari S,5 Baldini L,3 Federico M,2 Ambrosetti A,4 Angrilli F,3 Colombi M,3 Cortelazzo S,5 Orsucci L,9 Pogliani E,7 Pulsoni A,8 Rigacci L,9 Ambrosetti A,4 Angrilli F,5 Colombi M,3 Cortelazzo S,5 Rota-Scalabrini D,10 Stelitano C,11 Villivà N,8 Zinzani PL,12 Brugiatelli M,13 on Behalf of The Intergruppo Italiano Linfomi


Introduction: indolent non-follicular lymphoma (INFL) is a group of relatively frequent lymphoproliferative diseases. Nevertheless extended clinical and prognostic studies are still lacking. The IIL has performed a retrospective study on 373 aimed to assess their clinical behaviour and prognostic features. Patients and methods: patients included into the study were observed from 1988 to 1999 by 4 Italian cooperative groups and 1 single institution and they needed to have a diagnosis of small lymphocytic lymphoma (SLL), Immunocytoma/lymphoplasmocytic lymphoma (IC), marginal zone lymphoma (splenic or nodal, MZL) supported by histo-morphologic and phenotypic data. So far 357 patients (median age was 63 years, range 28-94; M/F ratio: 1.1) are evaluable and 16 were excluded due to incorrect date of diagnosis. Besides the usual clinical features, the following ones were also evaluated in survival analysis: anatomical sites of disease at diagnosis (nodal or bone marrow or splenic +/- bone marrow or disseminated disease) and presence of active disease defined as having at diagnosis at least one among Hb<11 g/dL, Plt <100×10^9/L, bulky disease, systemic symptoms and diffuse bone marrow infiltration. Results: 43% of cases were SLL, 17% LPL, 27% MZL and 13% indolent non follicular lymphoma unspecified. Advanced stage (III-IV) was present in 91% patients, bone marrow involvement in 78%, splenomegaly in 47%; 32% had 2 or more involved extranodal sites, 6% poor performance status (ECOG > 2), 16% B symptoms and 24% bulky disease. Involved sites of disease at diagnosis were: nodal 17%, bone marrow 6%, splenic 17%, bone marrow + nodal 36%, disseminated 25%; active disease was present in 53%. As far as laboratory data are concerned anemia (Hb <11 g/dL) was present in 23% cases, absolute lymphocitosis (Ly >5×10^9/L) in 14%, thrombocytopenia (plt <100×10^9/L) in 10%, ESR >30 mm in 40%, elevated b2 microglobulin in 52%, elevated LDH in 23%. A serum monoclonal component was detected in 30% of patients. Treatment varied among institutions and time of diagnosis, and consisted in: watch and wait policy in 32% and immediate treatment in 68%. Chemotherapy consisted in single agent (52%), CVP-like (5%), CHOP-like (37%) and Fludarabine (5%). With a median follow-up of 56 months (range 3-160), 3 and 5-years OS rates were 82% and 74% respectively. In univariate analysis the following 15 variables adversely affected overall survival (OS): age >60, stage III-IV, diffuse bone marrow involvement, B symptoms, anemia, elevated level of β2-microglobulin or LDH, ESR >30, serum albumin <3.5 g/dL, >1 extranodal sites of involvement and performance status, disseminated and splenic involvement and presence of active disease. Histological subtype and type of chemotherapy did not influence OS. Both International Prognostic Index and IIL model for follicular lymphomas were able to subdivide patients into three risk group with significant different OS rates (p<0.0001). Conclusions: This preliminary analysis on 357 patients with INFL showed that OS is affected not only by commonly used prognostic factors but also by the presence of parameters related to an active phase of disease. The final report including multivariate analysis will be presented.
EFFECT OF DEXRAZOXANE ON QT DISPERSION IN LYMPHOMA PATIENTS DURING ANTHRACYCLINE-BASED CHEMOTHERAPY
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In daily practice, onco-hematologists make use of parameters of systolic function (left ventricular ejection fraction or fractional shortening) to detect cardiotoxicity, but these methods are not able to identify acute cardiac damage. New methods, including the determination of QT dispersion, may be proposed to identify patients at risk of the development of early heart failure. Aim of the present study was to assess the effect of epirubicin-based chemotherapy (PROMECECytaBOM) on QT interval dispersion in 14 patients with aggressive Non-Hodgkin Lymphoma (NHL), and the effect of supplementation with dexrazoxane. The patients were randomly allocated to receive or not dexrazoxane clorhydrate (40 mg/m²) after epirubicin infusion. All the participants underwent 12-lead electrocardiogram (ECG) at baseline, after epirubicin infusion, and one hour later. QT intervals were measured from surface electrocardiograms and QT dispersion was defined as maximum QT - minimum QT occurring in any of the 12 leads. QT dispersion was corrected (QTc) for heart rate according with Bazett’s formula. All the patients showed increased QT dispersion (44.3±8.4 vs. 68.4±11.4 ms, p<0.001) and QTc dispersion (46.2±6.2 vs. 72.2±8.4, p<0.001) during epirubicin therapy. Patients who underwent supplementation with dexrazoxane clorhydrate exhibited a significant reduction of both QT (67.4±8.1 vs. 49.5±4.2 ms, p<0.001) and QTc dispersion (71.2±7.7 vs. 51.4±4.3 ms, p<0.001) if compared to placebo group (QT 69.3±7.6 vs. 64.2±6.9 ms; QTc 72.8±8.1 vs. 67.3±7.2 ms, ns). In conclusion anthracycline-based chemotherapy is associated with an increased QT interval dispersion which may be attenuated by dexrazoxane clorhydrate. Moreover, QT dispersion may represent a sensitive tool to identify the first signs of cardiotoxicity induced by anthracyclines and interferon therapy in the treatment of Hairy Cell Leukemia (HCL). In fact, both 2-CDA and DCF are able to elicit a complete or partial response of long duration in the vast majority of cases and with a very good toxicity profile. In addition re-treatment of patient with the same purine analog is effective in relapsed cases. Before the introduction of purine analogs, interferon therapy already demonstrated a high efficacy in terms of both response rate and survival, while splenectomy remained useful in a small minority of cases under because of uncommon clinical conditions. The present retrospective study is aimed to analyse the possible usefulness of interferon treatment in patients resistant to purine analogs by the presentation of three clinical cases. Case Report. Out of 53 HCL patients observed in our unit from 1982 to 2003, we successfully used interferon as salvage treatment in 3 cases. Case #1. A 66 years old man, diagnosed in 2001 as typical HCL according to morphological and phenotypic study and requiring immediate therapy because of pancytopenia and symptomatic splenomegaly. After first line treatment consisting of DCF no response was obtained. Thus, CDA therapy was administered, again without evidence of response. Finally, interferon was started at the standard dose of 3MU/day with gradual recovery from cytopenia and splenomegaly reduction. Interferon was discontinued after 4 months because of patient refusal, with progression of splenomegaly after IFN discontinuation. Case #2. In 2001, a 49 year old man diagnosed as typical HCL according to morphological and phenotypic study required immediate therapy because of severe pancytopenia and massive splenomegaly. After first line treatment consisting of DCF no response was obtained. Thus, CDA therapy was administered, again without evidence of response. Finally, interferon was started at the standard dose of 3MU/day with gradual recovery from cytopenia and splenomegaly reduction. Interferon was discontinued after 8 months because of a psychiatric comitant disorder, with persistent very good partial remission (VGPR) 6 months after IFN discontinuation. Case N.3. A 26 years old lady, diagnosed in 1997 as typical HCL according to morphological and phenotypic study and requiring immediate therapy because of pancytopenia and symptomatic splenomegaly. After first line treatment consisting of DCF no response was obtained. Thus, CDA therapy was administered, again without evidence of response. Finally, interferon was started at the standard dose of 3MU/day with gradual recovery from cytopenia and splenomegaly reduction. Interferon was discontinued after 4 months because of patient refusal, with progression of splenomegaly after IFN discontinuation. Case #2. 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ACUTE MYELOID LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES II

**P0205**
THE HDAC INHIBITOR D1 INDUCES APOPTOSIS AND DIFFERENTIATION IN A T(8;21) ACUTE MYELOID LEUKEMIA CELL LINE VIA MAPK MODULATION

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Acute myeloid leukemia (AML) is a disease characterized by a block of maturation. Genes coding for core binding factors are rearranged in a considerable subset of AML cases, and result in an altered interaction of CBF subunits with transcriptional co-regulators (NCOR/SMRT). Recruitment of HDAC is also altered in AML, and a subsequent transcriptional repression of target genes involved in myeloid maturation is determined. We recently demonstrated that sodium butyrate and the stable prodrug xylitol butyrate derivative (D1) as single drugs restore histone acetylation and granulocytic maturation in the t(8;21)-positive Kasumi-1 cell line, as well as primary CBF-AML blasts. These effects are paralleled by massive apoptosis as well as reduction of cell number. The mitogen-activated protein kinases (MAPK) have been shown to regulate a wide variety of cellular processes such as cell proliferation, differentiation and apoptosis. D1 induced ERK activation (peaks at 2-6h and 48-72h), D1 transiently activated p38, which was later suppressed. JNK was also activated following D1 treatment, with differences among the JNK isoforms. Specific inhibitors of the ERK, p38 and JNK pathways (PD98059, SB203580 and SP600125, respectively) were then used to investigate the role of MAPK in modulating the effects of D1 in Kasumi-1 cells. Inhibition of the ERK and p38 pathways did not seem to interfere with D1-induced apoptosis, as determined after 24 h by the annexin-V test. On the other hand, apoptosis was induced by D1 or SP600125 alone, while the treatment with both D1 and SP600125 induced an additive effect. These results were confirmed by western blotting evaluation of caspase-3 activation. Indeed, caspase-3 precursor decreased and the active caspase form appeared after a 24 h treatment with D1 and SP600125 alone, while the combination of D1 with SP600125 determined maximal effects. Caspase-3 activation was unchanged by the treatment with PD98059 or SB203580, alone or in combination with D1. D1 induced granulocytic differentiation as indicated by the significant increase in cells expressing both CD11b and CD15. PD98059 or SB203580 alone did not show any effect on maturation. SP600125 alone was able to induce significant differentiation and 50% mature cells were obtained with SP600125 plus D1. PD98059 partially prevented the D1-induced reduction of cell number, while was ineffective on D1-induced cell differentiation, suggesting that the ERK pathway is involved in the effects of D1 on proliferation rather than differentiation. SB203580 determined effects similar to those of PD98059 on cell number, and partial inhibition of maturation. It is worth pointing out that none of the above MAPK inhibitors interfered with histone acetylation which occurs after D1 treatment of these cells. The early modulation of MAPK suggest that D1 exerts its action also via HDAC-unrelated pathways as well as HDAC inhibition.

**P0206**
FLT3 MUTATIONS IN ACUTE PROMYELOCYTIC LEUKEMIA: INCIDENCE AND CLINICAL CORRELATIONS

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The t(15;17) translocation rearranges the PML gene with the RARα gene and creates the PML-RARα fusion protein on the translocated chromosome 17. That chimeric protein marks acute promyelocytic leukemia (APL) cells and play a crucial role in the pathogenesis of the disease. However, it has been reported that additional genetic changes may cooperate with PML-RARα fusion protein to generate the leukemic phenotype. Recently, some studies have pointed out that one gene which collaborate with the chimeric protein is FLT3. This last, which is a member of receptor tyrosine kinases, is expressed in leukemia as well as in normal hemopoietic cells. FLT3 is targeted by internal tandem duplications (ITD) or by D835 mutation in about 40% of acute myeloid leukemia patients and recent reports have demonstrated that it is mutated in about 10-37% of APL patients. Basing on these data we have decided to determine the frequency of FLT3 mutation in our APL patients and to establish if it correlates with any peculiar clinico-biological characteristics. Thirty-two APL patients, having diagnostic and follow-up samples, were analysed sequentially for the ITD of the FLT3 gene. Such a mutation was detected in six patients (18.7%). Cytogenetically all these patients presented only the classical t(15;17) chromosome translocation without any additional defect on karyotype analysis. FISH with PML and RARα specific probes confirmed the rearrangement in all of them. Clinically, the observation of FLT3/ITD was strongly correlated with an elevated peripheral white blood cell count (median WBC count in ITD positive patients 12x10^9/L (range 4-44) vs.
sus 2.7 (range 0.8-14) in FLT3 negative patients] and with a low fibrinogen concentration. Morphologically these patients more commonly presented a M3 variant, whereas we did not observe any association with any peculiar isoform of PM-L-RAR α fusion protein (a long isoform was detected in three cases, a short in two and a variable in one). A complete hematologic remission was achieved in all the patients without the mutation and in all patients but one harbouring the FLT3/ITD mutation. The five FLT3/ITD negative patients who relapsed achieved a second remission while one of those carrying the FLT3/ITD died of disease and the other one achieved a third remission. The follow-up samples of patients with persistent complete remission were constantly negative for FLT3/ITD, while those of patients experiencing relapse were positive for FLT3/ITD later than for PM-L-RAR α, probably because of a lower sensitivity of the RT PCR detecting the mutation. Our data confirm that FLT3/ITD are discovered in APL patients presenting a more aggressive disease on clinical diagnosis. However such a mutation does not seem to have an important role in leukemia progression.

PO207
INTENSIFIED REGIMEN (IDA-FLAG) COMPARED WITH STANDARD INDUCTION TREATMENT FOR ADULT AML
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Background: Despite progress in the treatment of acute myeloid leukemia (AML), overall survival is still poor and only 30% of patients will be cured. Allogeneic stem cell transplantation (alloSCT) performed during first complete remission (CR) is the therapy of choice for young patients, when a HLA compatible donor is available. For those lacking a donor, high dose chemotherapy plus autologous SCT may result in prolonged survival given that a ‘good quality’ CR is obtained before stem cell collection. Previous studies, concerning the pharmacology of cytarabine (ARA-C) and of its active metabolite ARA-CTP, have been the basis for the association of Ara-C and fludarabine, the last one as chemomodulator. Aim of the study: To evaluate whether an aggressive induction therapy could overcome the poor risk factors, resulting in an improvement of the rate and of the ‘quality’ of CR without negatively influence the feasibility of planned post remission therapy. Methods: between March 1998 and December 2002, 73 consecutive AML (non M3) patients aged less than sixty, received as induction one or two courses of Ida-FLAG: fludarabine 30 mg/m² days 1-4, cytarabine 2 g/m² days 1-4, idarubicin 12 mg/m² days 2-4, G-CSF sc from day -1 to 4 and from day 10 to granulocyte recovery. Early consolidation was been IdaFLAG (18 pts) or FLAG (42 pts). Thereafter, patients in CR were designed to receive as late consolidation allogeneic or autologous SCT according to risk factors and donor availability. Results: Median age was 46 years (range 25-61), M/F 31/42, 18 pts (25%) had a documented previous MDS. WBC median was 12.000/µL (range 2.000-138.000). Cytogenetic risk was low in 10%, high in 16%, intermediate in 57%, not valuable in the others. Overall, 60 (82%) patients achieved CR, 49 after one course. 9 (12%) had refractory disease and 4 died early or while aplastic (acute renal failure, cerebral hemorrhage, pneumonia, ileotiflitis). After consolidation chemotherapy, 13 underwent allogeneic and 15 autologous SCT, 4 are too early. All the others failed mobilisation, refused treatment or relapsed early. After a median follow-up of 23 months, the median survival of the whole group is 19 months, with a plateau of 41% at 30 months. Toxicity: The median time to recovery platelet >20x10¹⁰/L and granulocyte >1x10⁹/L was 23 and 22 days, respectively. The median red blood cells concentrates transfused per patient was 6 (range, 2-11). Median number of platelet apheresis transfused per patient was 5 (range 2-10). Twenty-eight patients had moderate bleeding, 4 severe. Fever were observed in 59 patients (80%). Infections were clinically documented in 10 patients, microbiologically documented in 25 and of unknown origin in 24. No patient developed congestive heart failure and neurological toxicity that could be attributed to anthracycline or to the association of fludarabine and Ara-C administration, respectively. Grade III mucositis was observed in 8 cases. Transient laboratory evidence of self-limiting hepatic dysfunction was observed in 15 patients (20%). None of the patients has shown a toxicity so severe to hamper the feasibility of planned post remission therapy. Comparing these results to our historical control (98 AML patients aged less than sixty treated with ICE in the immediately antecedent period) Ida-FLAG obtained a better rate of RC (82% vs 71%), mainly in the intermediate cytogenetic risk (88% vs 59%, p=.006). Although the overall survival at 30 months seems to be slightly better in the Ida-FLAG group (41% vs 33%), this benefit doesn’t lead to any improvement in the EFS (30% in both groups). Conclusions: an intensive induction regimen yields a better CR rate without hampering the post remission therapy. However, this does not translate in a substantial advantage on overall survival respect the historical control.
FLT3 is a member of the class III receptor tyrosine kinases expressed in leukemic cells, as well as in immature hematopoietic cells, playing an important role in stem cell proliferation and differentiation; it is the single most commonly mutated gene in acute myelogenous leukemia (AML). Two different types of FLT3 mutations have been identified, both resulting in constitutive activation of FLT3 receptor and induce factor-independent proliferation of hematopoietic cell lines. Internal tandem duplications (ITD) arising from duplications of the juxtamembrane domain have been found in 20-30% of AML patients and may adversely affect clinical outcome. Point mutations at codon 835 (D835), within the tyrosine kinase domain, occur in approximately 7% of AML and its clinical and prognostic relevance is not yet well defined due to the small number of patients studied. This study was designed to characterize the incidence and the potential prognostic impact of FLT3 mutations in 29 adult patients with acute promyelocytic leukemia (APL) at the time of diagnosis and in 2 relapsing patients. Genomic and message sequences of FLT3 gene, deriving from exons 14-15 (ITD), and exon 20 (D835), were amplified by single-step PCR. FLT3/ITD were found in 8 (27.6%) patients: the location and size of the duplications varied from sample to sample; D835 mutations were found in 5 cases (17.2%): 2 D835Y and 2 D835H. A silent mutation in R834 and a complete deletion of codons D835 and I836 (17.2%) 2 D835Y and 2 D835H. A silent mutation in R834 and a complete deletion of codons D835 and I836 (17.2%) was also found. Ongoing analyses are aimed to correlate the presence of the different types of FLT3 mutations with the clinical outcome evaluating them as a reliable predictive prognostic factor in APL patients.
patient who resulted positive at the molecular analysis is disease free after induction therapy at 7 months from diagnosis. Twenty out of 26 achieved CR, which lasted a median of 9 months (range 2-126). Thirteen of these 20 patients had a relapse. Median survival was 15 months (range 2-89). Conclusion. In conclusion the preliminary results of our study, which is still in progress, show a prevalence of FLT3 mutations that is comparable with that reported in larger series of patients. We did not find a clear correlation between FLT3 status and clinical outcome but we need to analyze more patients before drawing any definitive conclusion.

PO210
PRECURSOR AND MATURE ENDOTHELIAL CELLS ARE INCREASED IN THE PERIPHERAL BLOOD OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES
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Angiogenesis has been associated with vascularization during ovulation, placentation, and embryogenesis, but also with solid tumor growth, dissemination, and metastases. Recently, angiogenesis has been proposed as a prognostic indicator in many solid cancers, and it has been associated with myelodysplastic syndromes (MDS), chronic myeloid leukemia (CML) acute lymphoid (ALL), and myeloid leukemias (AML). In particular, increased circulating endothelial cells (CECs) have been associated with breast cancer and non-Hodgkin's lymphoma (NHL). The demonstrated increase of CEPs in the PB of MDS patients strengthens the hypothesis of a possible role of angiogenesis in MDS pathogenesis, as previously suggested in this disease by the MVD increase reported by us and others. This finding suggests that this role is present from the early phases of the disease, as no differences were detected between low and high risk groups.

PO211
THE ROLE OF FLOW CYTOMETRIC ASSESSMENT OF APOPTOSIS AND TELOMERE LENGTH IN MYELODYSPLASTIC SYNDROMES
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Flow cytometry is emerging as a useful tool in the assessment of the diagnosis and prognosis of patients affected by myelodysplastic syndromes (MDS). In the present work, we evaluated the role of flow cytometry in the characterization of hemopoiesis in 55 MDS patients (median age 74 years, 32 RA-RARS, 3 CMML, 10 RAEB, 10 RAEB-t). Apoptosis was studied in the CD34+ cell compartment by means of annexin V, anti-Fas and anti-Bcl2 monoclonal antibodies while telomere length was analysed by flow cytometry and fluorescence in situ hybridization (Flow-FISH) in peripheral blood cells. Apoptosis was significantly increased in IPSS low-int1 (n=39) in comparison to IPSS int2-high (n=16) patients.
Results. The percentage of annexin V- and Fas+ CD34+ cells inversely correlated with the percentage of bone marrow blast cells (r=-0.522, p<0.001 and r=-0.501, p=0.001, respectively) and the percentage of total CD34+ cells (r=-0.547, p<0.001 and r=-0.335, p=0.03 respectively). The telomere lengths in granulocytes and lymphocytes in MDS patients did not seem to decline significantly with age as observed in normal age-matched controls. In MDS patients, the telomere lengths in granulocytes were found to be significantly shorter than those in age-matched controls (p=0.004) while no significant difference was observed concerning lymphocytes. Granulocyte telomere lengths inversely correlated with the percentage of BM blasts (r=-0.2778, p=0.039). Finally, granulocyte telomere lengths were significantly shorter in IPSS int2-high than in IPSS low-int1 patients (p=0.008) and in patients with intermediate- unfavourable chromosomal aberrations than in patients with favourable cytogenetics (p=0.021). Overall, our results suggest that in low-risk patients hematopoiesis is mainly characterised by increased apoptosis while high-risk patients present less apoptosis but shorter granulocyte telomere lengths, which may favour the higher incidence of unfavourable cytogenetic findings and consequently the worse prognosis.

PO212
PIEMONTE MYELODYSPLASTIC SYNDROMES (MDS) REGISTER:
EPIDEMIOLOGICAL CONSIDERATIONS

Background. The incidence of myelodysplastic syndromes (MDS) is under-estimated by cancer registers, and the relationships between environmental risk factors and cytogenetic abnormalities or other biological markers are not well studied. Design and Methods. In 1999 the Piemonte MDS register was created thanks to the cooperation of both hematologic and internal medicine departments, with the purpose to collect new important epidemiological information. In patients we aimed: 1) to define homogeneous diagnostic guidelines; 2) to collect epidemiological information on risk factor exposition; 3) to correlate citofluorimetric and citogenetic data with the clinical behaviour of the disease; 4) to cryopreserve bone marrow cells for molecular biology studies. Results. From June 1999 to May 2003, 504 MDS patients were registered from 37 different institutions: 261 (52%) from hematology and 243 (48%) from internal medicine departments. Mean age was 72 (range 27-96), with the following distribution for class of age: 60 years 60 patients (12%); 61-70 years 139 patients (28%); 71-80 years 188 patients (37%); < 81 years 117 patients (23%). 296 patients (58%) were males. Information on age related comorbidity was available in 289 cases and only 27 patients (9%) did not show any other associated disease. 139 patients (48%) presented with comorbidity, while 82 (29%) and 41 (14%) showed respectively two and three other associated diseases. Anamnestic information on previous cancer and detailed data about former myelo-toxic treatments were available in 390 and 290 patients respectively, with the following incidence rates: previous cancer 22%; chemotherapy 10%; radiotherapy 11%; immunosuppressive therapy 4%. Cytogenetic was evaluated in 77% of patients. The distribution of the final diagnosis according to the WHO diagnostic groups is: RA 157 (33%), RA S 19 (4%), RCMD 33 (7%), RAEB I 98 (21%), RAEB II 86 (18%), 5q-syndrome 12 (2,5%), unclassifiable 28 (6%), CMML 40 (8,5%). The IPSS prognostic score was assessed in 342 patients: 97 patients (28%) had score 0; 159 patients (75%) had score 0.5-1; 50 patients (10%) had score 1.5-2; 30 patients (9%) had score < 2.5. The proportion of patients in whom bone marrow was ioplastic or presented increased fibrosis was 19%. Information about the exposure to pesticides was available in 317 patients with a high direct correlation: 66 cases (21%). Conclusions. MDS is a disease of old people and one or more other associated diseases are almost always present. Moreover a high proportion of patients is not followed by hematology departments. The percentage of MDS secondary to other neoplastic diseases is of about 20%, and a high correlation with pesticides exposure was observed. Further interview on environmental exposure to risk factors and correlation with chromosome abnormalities are useful.

PO213
DETECTION OF MYELOBLASTIN (MBN) GENE OVEREXPRESSION IN PATIENTS AFFECTED BY ACUTE MYELOID LEUKAEMIAS AND MYELODYSPLASTIC SYNDROMES
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Myeloblastin (MBN) is a serine protease with a broad spectrum of proteolytic activity. This gene is involved in the control of proliferation in myeloid leukemia cells and it confers factor-independent growth to hematopoietic cells when ectopically overexpressed in hematopoietic
cell lines. MBN has been found to be overexpressed in chronic myelogenous leukemias (CML) patients where it stimulates a specific T cell response (CTL). In order to study the role of MBN gene in the setting of acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) we investigated the expression levels of MBN transcript in 203 samples (151 BM and 52 PB) from 151 MDS patients, (89 RA, 41 RAEB, 18 secondary AML evolved from MDS, and 3 cases with 5q-), 40 samples (28 BM and 12 PB) from AML patients and 67 samples (15 BM and 52 PB) from healthy volunteers as control. In addition in several patients we tested the BM and PB samples during follow-up. All the AML and MDS patients were characterized at the cytogenetic, molecular and immunophenotypic level. The expression level of MBN was established on BM and PB samples using RT-PCR and quantitative Real Time PCR using a specific set of primers and probe (Assays-on-Demand, gene expression products, Applied Biosystems). The values obtained were normalized using ABL as housekeeping gene and the final results were expressed using the Ct method. We found that all the PB samples obtained from normal donors are negative for MBN expression by an RT-PCR evaluation. Accordingly using the RQ-PCR we detected very low levels of MBN mRNA. As expected, the BM samples from healthy volunteers, expressed higher MBN levels respect to the correspondent PB samples. By contrast the PB and BM samples collected from AML patients at diagnosis and from MDS patients expressed higher amount of MBN transcript respect to the normal controls. Moreover, by analyzing sequential samples during follow-up we found that, in patients who reached the clinical and molecular remission, the MBN transcript amount returned to the normal levels. Although further studies are required to better enlighten the biological significance of the overexpression of MBN gene in leukemic and myelodysplastic hematopoiesis, these data offer the possibility to have a marker of leukemic and myelodysplastic hematopoiesis available also in the peripheral blood, and it could probably identify an antigen to be targeted, as in the CML model, with a specific immunotherapeutical approach.

PO214
FARNESYLTRANSFERASE INHIBITORS INDUCE APOPTOSIS IN ACUTE MYELOID LEUKEMIA CELLS VIA ACTIVATION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND CASPASE-3 WITHOUT FAS, BCL-2 AND P53 MODULATION


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Mutations or overexpression of the Ras proto-oncogene have been described to play a role in the deregulated cell growth, survival and apoptosis of 20 to 30% of acute myeloid leukemias (AML). Ras is farnesylated by the housekeeping enzyme farnesyltransferase (FTase). This reaction is the first step in the signaling transduction pathway of Ras, enabling its linking to the plasma membrane, which is absolutely required for Ras oncogenic activity. FTase blockade has been recently utilized as a biochemical target for arresting the growth of tumors carrying ras mutations. We hypothesized that FTase inhibitors (FTI) effects in AML involve reversal of the apoptotic pathway inhibition. Consequently, we studied the effects of various FTIs (α-hydroxyfarnesylphosphonic acid, manumycin-A and SCH66336) on apoptosis and in vitro proliferation of the AML cell line KG1 and of primary cells from 50 AML patients, and investigated the mechanisms of their action by experimental blockade of various components of the apoptotic pathway. By allele specific oligonucleotide primer extension reaction, we also investigated the presence of oncogenic N-Ras and K-Ras mutations in primary cells from 40 patients with AML. By colorimetric MTT and methylcellulose clonogenic assays, we documented a dose dependent FTI-mediated inhibition of cell growth in KG1 and in 60% of AML. We detected oncogenic mutations of N-Ras in 27.5% of AML; by contrast, we did not observe K-Ras mutations in any AML cell sample. FTI-mediated inhibition of cell growth was observed in 80% and 70% of AML with and without mutations of N-Ras, respectively. By flow cytometry and DNA ladder analysis, we documented that the FTI-induced cytotoxic effect was in part related to enhanced apoptosis. However, Fas signalling was not involved, as Fas-receptor (FasR) and Fas-ligand (FasL) expression were not modified by FTI exposure, and there was no different susceptibility to FTI-mediated inhibition of cell growth based on Fas and FasL expression in CD34+ AML cells. Intracellular activation of caspase-1 and caspase-8 was also not altered by FTIs, and their blockade by caspase-1 inhibitor Z-DEVD-FMK and caspase-8 inhibitor IETD-FMK did not rescue FTI-treated AML cells. By contrast, after FTI exposure we observed activation of caspase-3 as assessed by flow cytometric detection of fluorescence derived from the cleavage of its specific substrate DEVD-AFC. Consequently, we studied the apoptotic transduction pathways which may activate caspase-3. No modulation of bcl2, bclxL, bclxS and p53 by FTIs was detected. Rather, FTIs increased inducible nitric oxide (iNOS) mRNA and protein levels, and this was associated with a higher NO production. In conclusion, our data document that FTIs may induce apoptosis both in Ras-mutated and non mutated AML via activation of inducible nitric oxide synthase and caspase-3, without evidence of FasR/FasL, bcl-2 and p53 modulation.
P0215
PROGNOSTIC IMPACT OF KARYOTYPE ON THE OUTCOME OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER HD-ARA-C PLUS IDARUBICIN, FOLLOWED BY FLAG+DAUNOXOME AND AUTOLOGOUS TRANSPLANTATION
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Elderly patients with ANLL have a poor prognosis mainly due to poor tolerance of aggressive chemotherapy and to the intrinsic disease resistance. Moreover, even after the achievement of CR, intensive consolidation with autotransplantation is feasible in very few cases. We used Amifostine as cytoprotective agent in addition to a regimen containing HD Idarubicine (40 mg/m²) and HD ARA-C (3 g/sm² for 5 days), the so-called Memorial. We treated 34 elderly patients (median age: 67, range: 56-78). Amifostine (740 mg/m² IV) was administered at day 3, followed by Idarubicin. Patients aged more than 70 yrs received 30% reduced schedule. Karyotype was normal in 18 pts, unfavorable in 11 pts, unevaleuable in 5 pts. We observed one toxic death due to infection. 25 out of the 34 evaluable responses were complete (65.5%), 83.3% (15/18) and 45.5% (5/11) in the intermediate and the poor prognosis cytogenetic groups respectively. Neutrophil (> 1500/µL) and platelet (>50,000/µL) recoveries were achieved at day 28 (range: 15-36) and 19 (range: 14-52) respectively. All pts experienced a grade III infectious episodes which responded to antibiotic treatment. Grade II-IV oral mucositis was never observed. Twenty-one patients underwent a consolidation course with FLAG + daunoxome (80 mg/m² i.v. day 1 and 2); 20 patients achieved a CR and 13 obtained a successful mobilization with a median collection of 6.6×10⁶ CD34+/kg (range 4-14). Twelve patients were successfully autotransplanted with a conditioning regimen including Busulfan 9 mg/m², melphalan 120 mg/m² in combination with amifostine and daunoxome 160 mg/m². Transplant related mortality was 25% (infectious complication in one patient, congestive heart failure in one case and severe mucositis in the other case). Hematologic recovery was complete in all patients: 12 days (range: 10-15) for neutrophil >1500/µL and 35 days (range: 15-35) for platelet >50,000/µL. Grade III-IV extrahematological toxicity was mainly due to mucositis (8%), and infections (33%). The sequence HD CHT and autologous transplant determined a final CCR rate of 27%, since 9 patients are alive and in CR with a median follow-up of 11.5 months (range 1-38); three relapsed patients are still alive while 22 patients died, 7 for treatment related causes (20%), 15 for disease progression. When patients were stratified by the cytogenetic prognostic group, a 50% of DFS at 32 months was achieved in patients with intermediate prognosis versus 0% in the unfavorable group. These results confirms the strong impact of Karyotype in the prognosis of AML elderly patients, which resulted to be significant even in a preliminary multivariate analysis exploring all the factors generally recognized as predictive of the DFS probability (age, FAB subgroup, karyotype subgroup, WBC Count at diagnosis, secondary or primary AML) with a 4.1 RR (p=0.02) in patients with poor karyotype in comparison with patients with an intermediate karyotype. The sequence of HD CHT and Autologous Transplant should therefore be reserved only to elderly patients with a favorable-intermediate prognosis karyotype, in who results seem to be not far from those of younger patients.

P0216
CLINICO-BIOLOGIC FEATURES OF ELDERLY PATIENTS (> 60 YRS) WITH ACUTE MYELOID LEUKEMIA: A UNICENTRIC STUDY ON 65 CONSECUTIVE PATIENTS
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From December 2001 to April 2003, sixty-five patients aged > 60 years with acute myeloid leukaemia were consecutively seen at the Department of Hematology of the University “La Sapienza” of Rome. At diagnosis, all patients were extensively characterized by a multiparametric approach, including immunophenotypic, cytogenetic and molecular analyses, to provide an extensive biological profile of this leukemic subset. Moreover, patients were further divided into two aged-stratified groups (I° group = age 60-70 yrs.; II° group age >71 yrs) to evaluate whether distinct biological features might be detected within these two groups. There were 33 and 32 patients in group I° and II°, respectively. Male/female distribution was 21/12 and 22/10. A previous myelodysplastic phase (MDS) was present in 11 (33%) and in 7 (22%) patients of the group I and II, respectively. The FAB classification observed in patients of group I° and II° was as follow: M0 - M2= 9/16 patients, M4 - M5= 17/11 cases while M6 leukaemia was diagnosed in one patient of group I°. A morphological FAB unclassifiable leukemia occurred in 6 and in 5 patients of group I and II, respectively. In the two patients groups the mean WBC cell count was 3.9×10⁹/L and 3.2×10⁹/L, the mean platelet count was 52.2×10³/L.
93.5×10⁹/L and 88×10⁹/L and the mean HB level (g/dL) was 9.3 and 9.4, whereas hemorrhagic symptoms with coagulopathy occurred in 3 and 1 patients, respectively. All these differences were not statistically significant. With regard to immunophenotype, combining the expression features of the CD117, CD34 and CD15 molecules, we defined the following 5 different AML subgroups: a) = CD117+ve, CD34-ve, CD15-ve; b) = CD117+ve, CD34+ve, CD15-ve; c) = CD117+ve, CD34+ve, CD15+ve; d) = CD117+ve, CD34+ve, CD15+ve, and e) = CD117+ve, CD34+ve, CD15+ve. The patients distribution between the two aged stratified groups respect to the immunophenotypic classes was as following: a= 2 and 3 cases in group I° and II°, respectively; b= 12/20; c= 3/5; d= 5/0 and e= 7/1. This distribution was statistically significant (p<0.001). Moreover, 54 patients (83%) had an evaluable karyotype. The distribution of the cytogenetic risk categories showed that a low risk karyotype (i.e.= t(15;17); t(8;21); inv(16)) was never demonstrated in any of the patients in the two age stratified groups; the intermediate cytogenetic risk category (i.e. normal karyotype, 11q23, +8, del(7) or other numerical/structural abnormalities) was detected in 26 and in 14 patients of group I° and II°, respectively; the poor risk category (i.e.= t(5;11), 5; del(5); -7, t(3;3) or inv(3), t(6;9), t(9;22) or complex karyotype) was present in 0 and 5 patients, respectively (p =0.2). Molecular analyses performed in 45 cases did not show any of the following alterations: BCR/ABL, AMML1/ETO, CBFB/MYH11 fusion genes and genomic ALL/MLL gene rearrangements. The FLT3ITD alteration was detected in 5 (15%) patients (group I° =3 patients; group II° = 2 patients) whereas the FLT3 D835 point mutation was detected in two additional cases. A statistical significant correlation was found between FLT3ITD and immunophenotypic classes (p =0.0002). As concern treatments 18 patients in group I° and 10 in group II° received an intensive chemotherapy versus 15 and 22 patients in group I° and group II° that received support/lows dose chemotherapy (p =0.07). The median actuarial survival was 240 and 412 days for group I° and II°, respectively (p =n.s.). In conclusion, to our knowledge, this is the first extended biologic characterization on a consecutive series of elderly AML patients. Our data showed that some heterogenic features could be distinguished within the two age stratified patients with respect to immunophenotypic and cytogenetic aspects. Further studies on larger number of patients should be performed to better categorize this biological heterogeneity in light of define those categories of elderly patients who could be really take advantage of intensive treatment.

Ablerrant methylation of normally unmethylated CpG islands has been documented as a relatively frequent event in immortalized and transformed cells and has been associated with transcriptional inactivation of defined tumor suppressor genes in human cancers. Cyclin-dependent kinase inhibitor p15INK4B (p15) is frequently inactivated by hypermethylation of the promoter region in many hematologic malignancies and especially in acute myeloid leukemias. In order to investigate the frequency of p15 gene promoter methylation in acute promyelocytic leukemia (APL), we used sodium bisulphite to obtain a complete conversion of all unmethylated cytosine to uracils, while 5-methylcytosines remain unaltered; then modified DNA was amplified by PCR with specific primers different for methylated (M) and unmethylated (U) DNA. In this study we evaluated a series of 33 patients with newly diagnosed acute promyelocytic leukemia (APL), observed in our Institutions in the period 1993-2003. According to FAB, 26/33 subjects (78.8%) were classical M3 and 7/33 (21.2%) were variant M3 (M3v). Molecular analysis of PML/RARα breakpoint was BCR1 in 18/33 (54.6%), BCR2 in 1/33 (3%) and BCR3 in 14/33 (42.4%). FLT3 mutations were present in 11/33 (33.3%) patients: 9/33 (27.3%) were ITD+, 3/33 (10%) showed D835 point mutation (in one patient both mutations were present). An abnormal methylation pattern was found in 6/33 (18.2%) patients: one of these patients only methylated DNA was detected, while in the remaining 5 patients both methylated and unmethylated DNA were amplified. By considering the 6 patients with p15 promoter methylation, 4 of them (66.7%) showed WBC higher than 10000/mcl; PLT were below 400000/mcl in 4/6 (66.7%); 5/6 (83.3%) were M3 and one M3v (16.7%); 3 (50%) had BCR1 breakpoint and 3 (50%) BCRT; 3 (50%) showed no alterations in FLT3 gene. 2 (33.3%) were ITD+ and one (16.7%) had D835 mutation. Then, p15 promoter methylation seems not to be correlated with any of these biologic and clinical features. All patients were treated according to GIMEMA AIDA protocol, based on chemotherapy + ATRA, with a 9 year follow-up, median 3 years. Relapses were observed in 4/33 (12.1%) patients, all of them with unmethylated p15 promoter. In the methylated
group 2 patients (33.3%) died early of hemorrhagic events; however, both patients were also with an high WBC count and FLT3-ITD+. The remaining 4/6 M patients (66.7%) are alive in CR. This study, although performed on a small number of patients, suggests that p15 promoter methylation status does not influence the prognosis in APL patients.

PO218
FLT3 MUTATIONS AND p15 PROMOTER METHYLATION ARE RARE IN THERAPY-RELATED LEUKEMIAS

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Therapy-related leukemia (tAL), occurring after exposure to chemo and/or radiotherapy, represents a serious event whose occurrence is more frequent than in the past. In the period 1996-2003, 17 patients (13 females and 4 males) were referred to our Istitution. When the primary malignancy was diagnosed the age was 14-67 years (mean 40, median 38); at the time of tAL diagnosis it was 16-72 years (mean 44, median 47).

Primary disorders were predominantly hematologic malignancies (15/17, 87%): Hodgkin’s (5/17), NHL (6/17), myeloproliferative disorders (2/17), AML (1/17) and MM (1/17); one patient had a breast cancer and another a rhabdomyosarcoma. For the primary malignancy 7/17 had been treated with CT alone (one of these had undergone to an autologous bone marrow transplantation); 10/17 patients had received both radio and chemotherapy. The different schedules of chemotherapy included alkylating agents (4/17) and topoisomerase II inhibitors (1/17) or both (12/17). After a median interval of 42 months (range 15-136) 15/17 pts tALL. Cytogenetic analysis was performed in 12/17 (70.6%): in 3/12 a normal karyotype was obtained, in 2/12 a t(15;17) alone was present, in 7/12 (58.3%) cases karyotype was complex. Within these latter 7 cases, most common clonal abnormalities involved chromosomes 5, 7, 8, 9, 21, 22, 13; together with other alterations, it was demonstrated a t(4;11) in one patient and an inv(16) in another one. In 15/17 patients (88.2%) FLT3 gene defects (both ITD in exon 11 and DB35 point mutation), leading to ligand-independent kinase activation, and methylation of the p15 tumor suppressor gene promoter, that causes its inactivation, were evaluated. We investigated the presence of FLT3-ITD by PCR on genomic DNA with fluorescent primers and subsequent capillary electrophoresis on the Abi Prism GeneScan 310. The DB35 point mutation was studied by digestion of PCR amplicons with EcoRV. To study the frequency of p15 gene promoter methylation, we used sodium bisulphite to obtain a complete conversion of all unmethylated cytosine to uracils, while 5-methylcytosines remain unaltered; then modified DNA was amplified by PCR with specific primers different for methylated and unmethylated DNA. We found only 2/15 patients with FLT3-ITD (to note, one was a patient with a normal karyotype) and no one with DB35 point mutation. Also, p15 methylation was an uncommon event; in fact only one out of 15 patients showed a methylated promoter. For the secondary leukemia all patients received a chemotherapy treatment; four of them also underwent to a bone marrow transplantation (2 autologous and 2 allogeneic BM T). The median survival after diagnosis of tAL was 13 months (range 1-80 months). At today, 7/17 patients are alive: the 2 patients with tAPL, who are in CR, 2 out of 2 with primary myeloproliferative disorders and 3/4 who received a BMT for the treatment of tAL. Our series confirms that t-AL has a dismal prognosis. Finally, although based on a small number of patients, this study could suggest that FLT3 mutations and p15 promoter methylation are rare events in tAL and perhaps other molecular alterations might support leukemogenesis.

PO219
FLUDARABINE, ARABYNOSIL CYTOSINE AND IDARUBICIN (FLAI), A COMBINATION OF NON MDR-RELATED DRUGS FOR TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA

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One of the most important causes of treatment failure in AML is the non specific multidrug resistance (MDR). This is mainly due to the overexpression of 170-Kd transmembrane glycoprotein (p170) which enhances the efflux of several drugs from the cells. The availability of agents which are not at all or minimally involved in the MDR efflux mechanism have recently allowed to test new therapeutic approaches in AML. We report here on the results of 138 newly diagnosed AML patients (FAB M3 excluded) treated with a com-
Combination of Fludarabine, Arabinosyl Cytosine and Idarubicin (FLAI) as first-line induction therapy. Patients responding to FLAI were scheduled for consolidation with a second course of FLAI or high dose cytosine arabinoside (HDAC), and autologous stem cell transplantation (ASCT) or allogenic BMT, when feasible. A complete remission after a single course of FLAI was achieved in 99/138 (72%) patients. The median time of granulocyte recovery (ANC>1.000/µL) was 24 days (range 15-55) and a platelet count > 50.000/µL was reached after a median of 22 days (range 15-80). The non-hematologic toxicity was mild and the most common side effects were nausea and vomiting (WHO grade I or II). In particular only 6/138 patients developed a grade 3 or 4 gastro-intestinal toxicity. Before therapy, the p170 expression in bone marrow blasts was evaluated in 95 patients (69%) by an indirect immunofluorescence method with the anti-p170 monoclonal antibody M-RK-16. The results were expressed as the mean fluorescence index (MFI). At present, no significant difference neither in the CR rate nor in the overall survival (OS) and disease free survival (DFS) was observed between the patients who were overexpressing the p170 glycoprotein (MFI > 6) and the patients who had lower p170 levels (MFI < 6). These results suggest that the FLAI regimen is a good first-line induction therapy for patients with newly diagnosed AML. Our results suggest that FLAI regimens can be useful to overcome primary MDR-Pgp mediated.

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P0220 CONTINUOUS SERIAL INFUSION OF FLUDARABINE AND CYTARABINE FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A PHASE II STUDY

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New approaches are needed for acute myeloid leukemia (AML) in elderly individuals. The combination of fludarabine (FAMP) with cytarabine (ARA-C)±G-CSF has been proven to be effective in poor risk AML. However, little is known as concerns continuous sequential infusion of the two drugs in newly diagnosed patients. We investigated the efficacy and toxicity of a regimen including FAMP + ARA-C administered as sequential continuous infusion (CI-FLA) in a series of untreated non-M 3-AML patients aged more than 60 years. FAMP was given at a loading dose of 10 mg/m2 over 15 min at day 0, and after three hours and half ARA-C at a loading dose of 390 mg/m2 over 3 hours were given; at the end, FAMP at 20 mg/m2/ci/24 hours for a total of 72 hours and ARA-C at 1440 mg/m2/ci/24 hours for a total of 96 hours were started. G-CSF was added at day +15 at a dose of 5 microg/kg. Patients achieving CR were programmed to receive an additional course of CI-FLA. Following consolidation, G-CSF at 10 µg/kg was given from day 15 in order to mobilize CD34+ cells. Between June 2001 and April 2003, 39 patients received the treatment. Median age was 69 years (range 61-78). In 13 patients (33%) an antecedent myelodysplastic syndrome preceded overt AML. Cytogenetic analysis showed normal karyotype in 16 patients, complex karyotype or other unfavourable chromosomal abnormalities in 15 cases, no mitoses in 8 cases. Finally, 34 patients were affected by one or more concomitant diseases requiring specific treatment. Overall, 26 (67%) patients achieved CR, all following one course of CI-FLA. There were 7 induction deaths (18%), all due to severe infections, while 6 patients (15%) were refractory to induction treatment. The median number of days to neutrophil > 0.5×109/L and platelet > 20×109/L was 18 (7-34) and 19 (9-26), respectively. Patients needed a median of 4 platelet units (1-15) and 6 blood units (2-17), respectively. All patients required broad spectrum empiric antibiotic therapy, while 7/39 cases (18%) needed intravenous antifungal treatment. Documented infections occurred in 6 cases (15%). Twenty-three patients out of 26 were eligible for the programmed consolidation course. Five of them died from infectious complications during subsequent pancytopenia; therefore, the last 19 patients received FAMP+ARA-C at 48 and 72 hours, respectively. Fifteen patients were monitored for the mobilization of CD34+ cells, collection being successful in 10 (67%). Median number of CD34+ cells/kg collected was 9.6×10^6 (2.4-42.7), the median number of apheresis was 2 (1-3). Overall, 7 patients have received autologous stem cell transplantation. After a median follow-up of 6 months (2-17), 18 patients are alive: among these, 14 are in continuous CR, while three have relapsed and one never achieved CR. In conclusion, this study demonstrates that CI-FLA is an effective and well-tolerated regimen for elderly patients with AML. Therapeutic results are extremely encouraging as to CR achievement and CD34+ cell collection. A longer follow-up is clearly needed to properly evaluate therapeutic results in terms of long-term disease-free survival.
PO222
IN VIVO PRIMING WITH RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR INCREASES THE EXPRESSION OF CD33 IN ACUTE MYELOID LEUKEMIA BLAST CELLS AND POSSIBLY ENHANCES THE CYTOTOXIC EFFECT OF GEMTUZUMAB-OZOGAMICIN: RESULTS OF A PILOT STUDY
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Gemtuzumab ozogamicin (GO, Mylotarg) has recently been approved for the treatment of CD33+ acute myeloid leukemia in elderly patients. Nevertheless, complete remission rates in these patients are less than 30%. CD33- blasts may escape killing by agents such as GO and this may be the reason for the lack of adequate response. In pre-clinical in vitro studies, we have shown that G-CSF increases the proportion of CD34+CD33+ cells, in particular of cycling blasts. To increase the intensity of CD33 antigen expression on blast cells, 4 elderly patients with relapsed AML were pretreated for 3 days with 300 mg lenograstin followed by Mylotarg 9 mg/m². The proportion of CD33+ and CD34+/33+ blasts increased in all patients following G-CSF. Three patients achieved complete remission (CR) after the first treatment course, while one patient obtained partial remission after the first course and CR after the second. At present, all patients are alive. Three of them are in continuous CR at 23, 12, and 8 weeks, while the patient who required 2 treatment courses to achieve CR, had an early relapse. These data show that pre-treatment with G-CSF may increase the efficacy of gemtuzumab ozogamicin targeted therapy, with mild and reversible extrahematologic toxicity.

PO223
COMBINATION OF LIPOSOMAL DAUNORUBICIN, FLUDARABINE AND CYTARABINE (FLAD) IN PATIENTS WITH POOR RISK ACUTE LEUKEMIA
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Background and Aims. Fludarabine combinations with Ara-C, G-CSF and Mitoxantrone or Idarubicin result in a well known synergistic enhancement of antileukemic activity and have been favourably employed in high risk AML. Daunorubicin is one of the most important cytotoxic agents in the treatment of acute leukemia (AL). Its use is usually limited by drug-induced cardiotoxicity depending on the cumulative dose administered. Compared to the unencapsulated free antracycline, liposomal daunorubicin (Daunoxome, DNX, Nexstar) is characterized by a higher tumor cell delivery, an improved pharmacokinetic and therapeutic indices, and therefore by a reduced toxicity profile. Design and Methods. We gave Fludarabine 30 mg/m² followed after 4 hours by Cytarabine 2 g/m² (infused in 4 hours) and DNX 100 mg/sqm (infused in 1hour) × 3 days (FLAD) to investigate the toxicity, safety and efficacy of the regimen in patients with poor risk AL. We have included at now 47 patients: 9 with refractory or relapsed acute lymphoblastic leukemia (ALL,one after allogeneic BMT, 6 with unfavorable karyotype), 35 with acute myeloid leukemia (AML) and 3 with CML in blastic phase. AML patients were administered FLAD for refractory disease (8), first or second relapse (11, three of which after autologous bone marrow transplantation) or as first line treatment (16 patients, of which 13 over 60 years of age and 5 with post- MDS AML). Overall median age was 60.5 years (range 13-76). Poor prognosis karyotypes have been detected in 13 patients. Results. FLAD was well tolerated in most patients. Major infections were observed in 6 patients (3 sepsis and 3 pneumonia). Neutrophil (N > 0.5×10⁹/L) and platelet (Plt > 20×10⁹/L) recovery required a median of 20 and 18 days from the end of therapy, respectively. As expected, non-hematologic toxicity was mild, and in particular no signs of cardiac toxicity have been recorded. The outcome is reported in the table. Three patient died before response evaluation of cerebral hemorrhage (2) and intestinal infarction (1). Twenty-one patients did not respond, of which all patients with refractory AML and CML in blastic phase. Six patients underwent allogeneic HLA matched stem cell transplant (3 M UD), one patients was submitted to autologous stem cell transplant. Conclusions. Our preliminary report shows that FLAD is a feasible and well tolerated treatment in poor prognosis AL. Its antileukemic activity is at least comparable to that exerted by mostly used conventional regimens. Furthermore this regimen does not preclude the feasibility of high dose therapy with autologous or allogeneic rescue.

Table 1. Outcome of patients treated with FLAD regimen.

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<tr>
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<th>AML (35pts)</th>
<th>ALL (43pts)</th>
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<td>First line</td>
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<tr>
<td>CR</td>
<td>10 (29%)</td>
<td>11 (13%)</td>
<td>0 (0%)</td>
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<tr>
<td>Median OS (mm)</td>
<td>6 (5-16)</td>
<td>8 (5-21)</td>
<td>9 (5-14)</td>
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<tr>
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<td>8 (5-12)</td>
<td>12 (9-23)</td>
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<td>Alive/dead</td>
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<td>2/1</td>
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PO224
FRAGILE X SYNDROME AND MYELODYSPLASTIC SYNDROME
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Myelodysplastic syndrome (MDS) is usually observed in elderly patients but it is rare in children and adolescents. When it occurs in young people it's often associated with congenital syndromes: Down syndrome, neurofibromatosis type 1, Fanconi's anemia, severe congenital neutropenia, Shammah syndrome, Noonan syndrome. We describe a case of Fragile X [Fra (X)] syndrome and MDS in a young boy. The patient was a 19 years old male with mental retardation whose pathogenesis had not been clarified. He was referred to our Department because a one month history of neutropenia, thrombocytopenia, oral facial herpes simplex infection, splenomegaly. A complete blood count demonstrated an absolute neutrophyl count of 109/L, platelet count of 75 x 10^9/L, LDH 896 times and abnormal hypermetilation within a coding region of a gene designed FMR-1 located on Xq 27,3. This mutation is frequent but the phenotype is variable and sometimes poor recognizable especially in females.

References

PO225
HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER INTENSIVE CHEMOTHERAPY IN HIGH RISK MYELODYSPLASTIC SYNDROMES AND SECONDARY ACUTE MYELOID LEUKEMIA. AN INTENTION TO TREAT ANALYSIS
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Background: Patients with HR-MDS and sAML have a median survival of 4-14 months. Allogeneic stem cells transplantation (AlloSCT) is the therapy of choice for younger patients with an HLA-compatible related donor (donorM MATCH). Non-myoeloblastic AlloSCT is an experimental option currently under evaluation for older pts (>55-60 years old). High dose chemotherapy with autologous SCT (ASCT) rescue is advisable in patients without a donorM MATCH. Obtaining CR before transplantation by means of intensive chemotherapy improves DFS after AlloSCT and permit to collect autologous putative polyclonal hematopoietic stem cells for subsequent ASCT. Since 1999 we have been treating HR-MDS and sAML pts up to 70 years old with an intensive program comprising induction chemotherapy and subsequent SCT transplantation. Aims: to retrospectively evaluate the feasibility of our program based on a intent-to-transplant policy. Methods: Between 1/1999 and 3/2003 37 newly diagnosed MDS/sAML pts entered our program. Median age: 56.5 (22-69), pts >59 yrs =12. Ten pts (27%) had a donorM MATCH, 26 had a familial partially compatible donor (HAPLO), 1 pt had no relatives. All patients have been initially treated with the FLAG-IDA regimen. Twenty-five pts received a second cycle: 12 FLAG-IDA, 3 FLAG, 1 FLAG+liposomal daunorubicin, 4
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FISH IDENTIFIES NEW CHROMOSOME ABNORMALITIES IN MDS/AML PATIENTS HAVING A COMPLEX KARYOTYPE
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A chromosome pattern consisting in more than three chromosome abnormalities or in more than three chromosome breakpoints, i.e. a complex karyotype, is detected in 15-20% and in 50% of patients with de novo and secondary MDS/AML. Such a cytogenetic picture is always associated with an adverse clinical outcome: a complete remission is achieved in 21-48% of patients and median overall survival is 1-5 months. However, despite its poor prognostic significance, a comprehensive analysis of patients having a complex karyotype has never been reached because of the important drawbacks of conventional cytogenetics (CC). Recently, multiplex-fluorescence in situ hybridisation (M-FISH) has demonstrated that such MDS/AML patients may harbour new distinct chromosome rearrangements, which identify novel prognostically different patient categories within karyotypically complex MDS/AML. Therefore, considering M-FISH data, we have performed FISH with whole chromosome painting probes or with probes exploring genes and polymorphic loci mapped within chromosome bands which were found to be involved in the structural defects present in 28 karyotypically complex MDS/AML. Our study was aimed at establishing the incidence of those new chromosome translocations, at identifying new cytogenetic aberrations and at defining the prognostic impact of such defects. Up to now we have analysed a total of 28 AML patients. The complex karyotype was at first identified by CC and subsequently defined by FISH. This last was carried out on mitotic figures from cytogenetic preparations. We used the LSI EGR1 (5q31), LSI D7S486 (7q31), LSI MLL probes and painting probes for chromosomes 1, 3, 4, 6, 7, 11. In order to distinguish chromosome 5 or 7 deletions from cryptic translocations FISH firstly used probes for EGR1 and D7S486 respectively and then the painting probes specific for either chromosomes. As expected, an interstitial or a terminal deletion of the long arm of chromosome 5 (5q-) was the most common chromosome defect having an incidence of 89%. A cryptic translocation targeting 5q was observed in a total of five patients, however none of them showed the cryptic t(4;5)(q31;q31) translocation. An iso(5p) was detected in another patient. Interestingly 28% of patients with 5q structural abnormalities showed a 17p deletion and two of them, both classified as M2, also presented a MLL amplification. This datum points to a possible cooperation among a possible tumor suppressor gene on 5q, TP53 and MLL. In addition, 5q- patients harboured other deletions, suggesting a possible molecular heterogeneity, which might probably translate into a different clinical outcome. Structural defects of chromosome 7 occurred at an incidence of 53%. Considering this last chromosome it was most frequently involved in unbalanced translocations with number 17 which was deleted within the short arms in a region located more centromerically to TP53. Monosomy 7 was the other most common defect. In two patients we observed a fragmentation of chromosome 7. MLL was amplified in two patients, translocated in one and duplicated in another one; all the other 24 patients showed a normal pattern. Chromosome 3 was targeted by a complex three-way translocation in only one patient. No defects targeted chromosomes 1 and 6. From a prognostic point of view a longer overall survival was seen in patients without MLL amplification and in those having no more than two defects targeting chromosomes 3, 5, 6 and 7. In con-
The t(1;7)(p11;q11) translocation is a rare chromosome rearrangement observed in myelodysplastic syndromes (MDS), acute myeloid leukemia (AML) and chronic myeloproliferative disorders. Usually, half of the MDS/AML patients carrying the translocation have been previously exposed to environmental carcinogens or to chemotherapy for another cancer. The incidence of the rearrangement is 2% in de novo MDS/AML and 3-7% in secondary disorders. The t(1;7) translocation is usually unbalanced and determines a trisomy for the long arms of chromosome 1 (+1q). In addition, FISH studies have demonstrated that the rearrangement should always be formally defined as dic(1;7)(p11;q11). In the period January 1990-December 2001 352 patients with de novo and 53 with secondary MDS have been cytogenetically studied at our Institution. A t(1;7) translocation, determining a +1q and always defined as dic(1;7)(p11;q11) by FISH studies, has been detected in 13 patients (3.7%). Patients' median age was 65 years (range 41-73) and they were six males and seven females. Seven patients, who previously suffered for another cancer, had been treated with alkylating agents for a median time of 70 months (range 36-168). Considering all the 13 patients two were classified as refractory anemia (RA), three as RA with excess of blasts (RAEB), three as RAEB in transformation (RAEB-t) and five as AML evolving from MDS. Four cases showed a marrow fibrosis on bone marrow biopsy. Considering the eight MDS patients an evolution in a more advanced MDS occurred in one RA patient, while an AML progression happened in three patients. Three out of the 13 patients did not receive any treatment, the remaining ten underwent different chemotherapy regimens. A complete hematological remission lasting ten months was reached in only one AML patient; all the others did not respond to chemotherapy. In our series median survival was 9 months (range 2-36). Our data confirm that an unbalanced t(1;7) rearrangement is a rather rare karyotype defect, which occurs more frequently in secondary MDS/AML. As recently suggested, in these last patients the alkylating agents used might have determined dicentric chromosome formation through a demethylation of the centromeric regions. Our series confirms that the rearrangement is correlated with an adverse clinical outcome.

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TRANSLOCATION T(1;7)[P11;Q11] IN MYELODYSPLASTIC SYNDROMES: INCIDENCE AND CLINICO-BIOLOGICAL FEATURES. IN MYELODYSPLASTIC SYNDROMES
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Trisomy 8 is a common acquired somatic mutation in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), which is associated with a not favourable prognosis. The anomaly may also occur as a constitutional mosaicism, which is a relatively rare condition associated with high phenotypic variability, including multiple anomalies and mental retardation. In few cases, the phenotypic features may be quite subtle, in the presence of normal or near normal IQ. A constitutional trisomy 8 mosaicism (CT8M) is usually detected in PHA-stimulated peripheral blood lymphocytes (PBL) or in cultured skin fibroblasts. In some patients the mosaicism is present in both these tissues, whereas in others it is apparently confined to either the blood or the skin. Recent evidences have indicated that trisomy 8 found in hematologic malignancies can sometimes derive from CT8M; in such cases the prognostic significance may differ from that of the cases acquiring the anomaly during the development of the neoplastic disease. In 5 patients with MDS (2 cases), AML post MDS (1 case) and de novo AML (2 cases) showing +8 on bone marrow (BM) cells at diagnosis, we performed cytogenetic analysis on PHA-stimulated PBL in order to verify the acquired or constitutional nature of the anomaly. In one case, the karyotypic analysis was also done on fibroblasts cultured from a skin biopsy. Chromosome investigations were performed according to standard methods by conventional analysis and interphase FISH, using a chromosome 8-specific pericentromeric probe (D2Z8; Institute for Human Genetics, Rotterdam, the Netherlands). CT8M was diagnosed in 1 MDS patient on the basis of the identification of +8 both in 5% of PHA-stimulated PBL and in 7% of skin fibroblasts. In a AML post MDS case, the trisomy 8 was detected in 6% of the PHA-stimulated PBL, suggesting
the constitutional nature of the anomaly. Both patients
did not show apparent signs of trisomy 8 syndrome. Trisomy 8 was not found on PB cells in the remaining
3 investigated cases. The study hereby reported indicates that CT8M may be a not unusual finding in
patients with hematologic malignancies and +8 on BM
cells, as we identified the abnormality in 2/5 investi-
gated cases. The detection of CT8M may be of impor-
tance, considering that the presence of the anomaly in
the leukemic clone may be due to chance and, in
absence of additional chromosomal changes, it may
bear the same prognostic value as a normal karyotype.
Our data suggest that occurrence of CT8M, even with-
out any obvious physical stigmata, should be investi-
gated in all MDS/AML patients with clonal trisomy 8 in
BM cells, in view of a correct clinical management of
these patients.

PO230
PROGNOSTIC FACTORS IN CHRONIC MYELOMONOCYTIC LEUKEMIA:
ANALYSIS OF 83 PATIENTS FROM A SINGLE INSTITUTION
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Chronic myelomonocytic leukemia (CMML) is a rare
clonal hematologic disorder mostly occurring in elder-
ly patients, characterised by 5-20% blasts in the bone
marrow and increased monocytic cells (>1000/mm³) in
the peripheral blood. We retrospectively analysed pre-
senting clinical features of 83 patients with CMML
diagnosed at our Institution and evaluated clinical-
prognostic significance of patients stratification in
myelodysplastic (MD) and myeloproliferative (MP) sub-
types on the basis of WBC count, as proposed by FAB
group. According to the stated cut-off of WBC count
of 13×10⁹/L, we identified 46 cases (55.5%) as having
dysplastic (MD) and 37 (44.5%) as having proliferative
(MP) variant. Main differences between the two groups
were in median survival, that was 20 months for MD
type and 17.4 months for MP type (p=0.02) and in dis-
ease progression rate, that was 29.7% for MP type and
15.2%, for MD type (p=0.01). By comparing the two
subgroups of patients, significant differences were
observed regarding gender (p=0.02), whereas median
age was not differing. Splenomegaly was present in
32.6% of MD patients and in 48.9% of MP patients
(p= NS); hepatomegaly was detected in 52% of MD
patients and in 64.8% of MP patients (p= NS). With
respect to laboratory findings at time of diagnosis, sig-
nificant differences were evident as to WBC, neutrophil
and monocyte counts, with the absolute neutrophils
and monocyte numbers being higher in the MP group.

Differences in hemoglobin levels and platelet counts
were slightly significant between the two groups,
whereas creatinine levels showed no significant differ-
ences (p= 0.057). From multivariate analysis, param-
ters associated with shorter survival were WBC count
> 13×10⁹/L and bone marrow trilinear dysplasia; fur-
thermore, the only significant factor for risk of acute
leukemia transformation was blast cells percentage
> 5% in the bone marrow. The application of different
prognostic scoring systems (Bournemouth, modified
Bournemouth, Sanz score and IPSS, this latter applied
to MD type only)showed a validity as indicator of short
survival for intermediate and high risk patients. Our
analysis provides support to the clinical utility of seg-
grating CMML into MD and MP variants on the basis
of WBC count. However biological and pathogenetic
aspects remain to be furtherly explored, which might
help for proper classification refinement.
Alemtuzumab (anti-CD52; Campath-1H) depletes both host and donor T cells when used in preparative regimens for allogeneic transplantation. This promotes engraftment even after nonmyeloablative conditioning and limits graft-versus-host disease (GVHD) even after unrelated or major histocompatibility complex (MHC) disparate allografts. We have examined the recovery of two distinct dendritic cell populations (myeloid, dendritic cell type 1 and lymphoid, dendritic cell type 2) after non-myeloablative conditioning with or without Alemtuzumab and have evaluated the kinetics of dendritic cell chimerism in both subsets. We have studied 17 patients with various hematologic and non-hematologic malignancies. Median follow-up was 170 days. Six patients underwent a nonmyeloablative conditioning regimen without Alemtuzumab, six received Alemtuzumab at 7.5 mg/m², two patients received it at 15 mg/m² and three patients received an aplo-identical graft after nonmyeloablative preparative regimen with Alemtuzumab at 30 mg/m². Fourteen patients infused unmanipulated peripheral blood hematopoietic stem cells and three selected CD34 peripheral blood stem cells. All patients engrafted. Flow cytometry assays were used for detection and evaluation of the overall median proportion of dendritic cell subsets. Dendritic cells were identified as negative for lineage markers and positive for HLA-DR expression: myeloid dendritic cells expressed CD1c=BDCA-1, CD11c bright, CD123 dim, CD45RO, while lymphoid dendritic cells were BDCA-4, BDCA-2, CD123 bright, CD45RA positive and CD11c negative. Peripheral blood samples were collected at defined intervals after transplantation. Dendritic cells were isolated by a multistep procedure: peripheral blood mononuclear cells were isolated by Hypaque-Ficoll density gradient centrifugation; dendritic cell fractions were then enriched by immunomagnetic bead selection process. BDCA-1 dendritic cell isolation kit includes CD19 microbeads for depletion of B-cells prior to the enrichment of CD1c (BDCA-1)-positive dendritic cells. BDCA-2 or BDCA-4 dendritic cells are directly isolated by magnetic labeling and positive selection. The goal of the selection was to obtain a final purity over 90%, in order to evaluate lymphoid and myeloid dendritic cell chimerism. The relative contribution of donor and recipient was determined by quantitating, in DNA preparation, informative microsatellite short tandem repeat alleles. Our data show a significant difference in the recovery of BDCA-1 dendritic cells between patients receiving Alemtuzumab and patients receiving a conditioning regimen without Alemtuzumab (p 0.02 no-Alemtuzumab versus Alemtuzumab 7.5 mg/m², p 0.04 no-Alemtuzumab versus Alemtuzumab 30 mg/m²). The difference remains statistically significant until 90 days after transplantation. Concerning with BDCA-2 subset, the difference is not yet statistically significant (p 0.06), but this result may be influenced by the limited number of patients analyzed. Comparing the number of BDCA-1 and BDCA-2 positive dendritic cells in the grafts and the number of CD34 positive cells infused, no significant differences are shown among the different groups of patients. We have compare the immune recovery between patients treated with Alemtuzumab 7.5 mg/m² and those that did not received Alemtuzumab: CD3/CD4 lymphocyte counts were lower with the use of Alemtuzumab and the difference resulted statistically different at 30 days after transplantation (p 0.0089). Chimerism analysis on myeloid and lymphoid dendritic cells has been performed on 10 samples: by day +30 more than 95% of dendritic cells were of donor origin.
Peripheral blood (PB) was the preferred source of repopulating hematopoietic SC. Comparison of these 50 patients with 34 patients who received an allogeneic bone marrow (BM) transplantation before 1996 (group B) revealed that the two groups were well balanced with respect to the main characteristics of the disease. In comparison with group B, patients in group A had a significantly longer overall survival (OS) (6-year projected: 27% vs 60%, respectively; p = 0.007), event-free survival (EFS) (6-year projected: 18% vs 39%, respectively; p = 0.004) and time to relapse (6-year projected: 41% vs 73%, respectively; p = 0.05). Overall, the transplant-related mortality rate was 42% before 1996 and 25% since that date. The rate of complete remission did not change appreciably over time, averaging approximately 40% on an intent-to-treat basis. As expected, the frequency of chronic GVHD with the use of PB was much higher than that observed with BM (62.5% vs. 30%, respectively). A multivariate Cox regression analysis of risk factors for outcome identified the more recent transplant period (after 1996) and less advanced clinical stage (I+II) at transplantation as the most important and independent variables favorably influencing both OS and EFS. It is concluded that major advances in the outcome of allogeneic stem cell transplantation for MM using myeloablative conditioning regimens were seen over the last years. Whether the improved outcome was, or not, partly due to the use of PB remains high, in the 25% range, a finding that makes the procedure not applicable on a standard basis to all young patients who have an HLA-identical donor. Ideal candidates to receive allogeneic transplantation are high-risk patients, as identified according to routine parameters and chromosomal alterations, who are likely to not benefit from autologous transplantation.

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HAPLOIDENTICAL T DEPLETED PERIPHERAL BLOOD AND STEM CELL PLUS BONE MARROW TRANSPLANTATION FROM MOTHER TO CHILD WITH THALASSEMIA DISEASE.

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Approximately 60% of thalassemic patients are not suitable for “gene therapy”, with the insertion of one allogeneic HLA identical stem cell into the empty bone marrow as the vector of the normal gene for β globin chain synthesis. We studied the use of the haploidentical mother as the donor for the stem cell, assuming that the immuno-tolerance established during the pregnancy would help bypass HLA disparity during the haploidentical transplant. We have employed a new preparative regimen to transplant in seven patients with thalassemia using T-cell-depleted three HLA allele disparate related peripheral blood stem cell (PBSC) plus bone marrow (BM) transplant. The median age of the patients was 5 years (range 3-8). All patients received hydroxyurea 60 mg/kg and azathioprine 3 mg/kg from day -59 until day -11, fludarabine 30 mg/m² from day -17 to day -11, busulphan 14 mg/kg starting on day -10, and cyclophosphamide 200 mg/kg, Thiotepa 10 mg/kg and ATG Sangstat 2.5 mg/kg, followed by a CD34+ T-cell depleted (CliniMacs sistem), granulocyte colony stimulating factor (G csf)mobilized PBSC from their HLA haploidentical mother. The purity of CD34+ cells after MACS sorting was 98-99%, the average number of transplanted CD34+ cells was 15.4x10⁶/kg and the average number of infused T lymphocytes from BM was 1.8x10⁹/kg. The patients received cyclosporin after transplant for graft versus host disease (GVHD) prophylaxis. Two patients rejected the transplant and are alive with thalassemia. One of the seven patients received a second transplant using purified CD34+ cells from his father, after the same preparative regimen resulted in a complete hematopoietic reconstitution and freedom from thalassemia. Five or the seven patients are alive and disease free with a mean follow-up of 11 months (range 6-18). This preliminary study suggest that the transplantation of megadose of haploidentical CD34+ cell from the mother is a realistic therapeutic option for those thalassemic patients who do not have genotypically or phenotypically HLA identical donor.
PO234
ANALYSIS OF HHV-8 INFECTION AND ASSOCIATED POST-TRANSPLANT DISEASES IN ITALIAN BONE MARROW/PERIPHERAL BLOOD STEM CELL DONORS AND RECIPIENTS (GITMO STUDY)


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Despite the fact that the Kaposi sarcoma is very rare after allogeneic/autologous bone marrow (BM)/peripheral blood stem cell (PBSC) transplant, other complications associated with human herpesvirus-8 (HHV-8) infection may occur in such patients, at least in Italy, where the seroprevalence of HHV-8 infection among blood donors is about 19%. We judge it appropriate to screen for HHV-8 infection, both by serology and polymerase chain reaction (PCR), in a large number of Italian donors and recipients of BM/PBSC, (provided by the GITMO: Gruppo Italiano Trapianto di Midollo), in order to assess the number of patients at risk for developing HHV-8 associated diseases. We have collected unseparated peripheral blood and serum samples from 126 pairs of donors/recipients of BM/PBSC. These samples have been collected from the recipients at time of transplantation, at 6 and 12 months after transplantation. Fourteen out of 126 BM/PBSC donors (11%) resulted positive for HHV-8 sequences. 14 out of 126 BM/PBSC transplant patients tested positive for HHV-8 by PCR (11%) at the time of transplantation, while 6 patients resulted HHV-8 positive after transplantation (5%). Analysis of samples at 12 months is ongoing. Of interest, one out of the latter 6 patients had received the BM/PBSC transplantation from a HHV-8 positive donor, and developed clinical manifestations (fever, rash, diarrhoea and hepatitis) at day 90 after transplant. By monitoring HHV-8 infection by Quantitative-PCR, we were able to attribute the clinical manifestations to HHV-8 primary infection in this case. In conclusion, we show, for the first time, that HHV-8 may be transmitted from a donor through a BM graft, although primary infection with HHV-8 in BM recipients is due to horizontal transmission in the majority cases. The screening and monitoring of HHV-8 infection may be considered in the setting of BM/PBSC transplantation.

PO235
AUTOGRAPHING FOLLOWED BY LOW DOSE TBI BASED NON MYELOABLATIVE ALLOGRAFTING IN MULTIPLE MYELOMA: THE GITMO EXPERIENCE


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The curative potential of allografting for myeloma (MM) patients has not been realised because of the high transplant-related mortality (TRM) associated with conventional allografting. To date, the best reported analysis (by the EBMT registry) showed a TRM of 21% at 6 months with a 55% 3-year survival for patients transplanted between 1994-1998. However, the median age in this cohort was only 44 (range 18-57) years whereas the median age of MM patients at diagnosis is approximately 65. In the attempt to reduce TRM and increase the eligible age for transplant, we are conducting a multi-center trial employing a tandem transplant approach for newly diagnosed stage IIA-IIIB MM patients up to the age of 65. Briefly, after induction chemotherapy, patients undergo G-CSF mobilized autografting with high dose melphalan (200 mg/m(e)2) followed, 2-4 months later, by low dose (20 Gy) total body irradiation, PBSC infusion from HLA-identical siblings, and immunosuppression with mycophenolate mofetil (15 mg/kg BID) for 28 days and cyclosporin (6.25 mg/kg BID) for a minimum of 80 days. To date, 62 patients (median age 55, range 34-65) have entered the study. Fifty-two patients have so far completed both transplant procedures. Allografts were carried out at a median of 75 (range 44-195) days after autografts. All patients readily achieved sustained donor engraftment. After a median follow up of 268 (30-1116) days post allografting, overall survival is 83% (43/52). The overall response rate evaluated in 45 patients (with a follow up of at least 84 days) is 87%, with 60% (27/45) complete (CR) and 27% (12/45) partial remissions (PR); disease progression was observed in only 1 (2%) after obtaining an initial PR. Remarkably, in 35/45 (77%) patients who were not in CR at allografting, 17/35 (48%) patients attained CR at a median of 90 days (range 28-180) showing a potent, though gradual, graft versus myeloma effect. Twenty-seven% of patients with a follow up of at least 28 days developed grade II acute graft versus host disease (GVHD) and 12% grade III-IV GVHD. In 41 patients with a follow up
PO236
EXTRACORPOREAL PHOTOCHEMOTHERAPY IN GRAFT-VERSUS-HOST DISEASE TREATMENT. SINGLE CENTRE EXPERIENCE IN 32 PATIENTS AFFECTED BY CHRONIC GVHD
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Chronic GVHD affects 30-60% of long-term survivors of hematopoietic stem cells transplantation recipients and is a major cause of morbidity and mortality. Standard GVHD treatment is unsatisfactory because poor results and toxicity. Extracorporeal photopheresis (ECP) has been demonstrated to be effective in chronic GVHD treatment. We studied 32 patients who had received HLA-matched stem cell transplants for hematological malignancies, from sibling (IS) (n = 23) or unrelated (UD) (n = 9) donors, 20 bone marrow transplants, 11 peripheral blood stem cell transplants and 1 DLI after relapse in UD. 15 were early disease and 17 advanced stage. Diagnosis were CML 8, AM/L/SMD 11, ALL 6, MM 4, CLL 1 and LNH 2. Transplant procedure was performed as elsewhere described. GVHD prophylaxis was done in all patients with Ciclosporine and Metotrexate, plus ATG in UD transplants. Thirty-two affected by extensive cGVHD patients were studied. 15 de novo cGVHD, 3 quiescent form and 14 evolution from aGVHD. (skin 24/32 pts, liver 13/32 pts, oral sicca 15/32 pts, ocular sicca 10/32 pts, bowel 7/32 pts, lung 8/32 pts, myositis 3/32 pts, thrombocytopenia 4/32 pts, serositis 2/32 pts and contracture 2/32 pts). At the start of ECP all pts. were refractory at least two lines of treatment: ciclosporine (28)/Tacrolimus(4) 32/32, steroids 32/32, azathioprine 10/32, thalidomide 5/32, micofenolate mofetil 6/32, Cyclophosphamide 2/32. After a mean of 17 cycles of ECP (range 1-46), cGVHD resolved completely in 10/32 (31%) and partially in 15/32 (47%). 7/32 patients (22%) did not respond to treatment, two experienced stable disease, two died for relapse and two died because progressive cGVHD. The best results were observed in skin, liver, bowel, lung and thrombocytopenia. A further benefit gained from the ECP treatment was that all responding pts were able to reduce the immunosuppression, that has been discontinued in 4 cases. Karnofsky performance score improved from 66% (range 20-90%) to 84% (range 60-100%). We also compared the results of pts who started ECP early versus those who started treatment later. Our study demonstrate a 50% of complete response if the ECP was started within six months, even if we have obtained a 67% of partial response within 12-58 months. No severe side effects were documented. Our results suggest that ECP is a safe therapy for extensive chronic GVHD resistant to conventional treatment, that it must be started early and continued until best response.

PO237
OUTCOME OF 131 CONSECUTIVE PATIENTS WITH AN HIGH-RISK HEMATOLOGIC DISEASE SEARCHING FOR AN UNRELATED DONOR
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Background. The aim of the study was to retrospectively evaluate the outcome of unrelated bone marrow donor searches for 131 consecutive patients initiated on February 1997 through April 2003. Design and Methods. At the time of search activation, 77 (58.5%) patients were affected by acute leukemia (AL), 24 (18.5%) by chronic myeloid leukemia (CML), 19 (14.5%) by lymphoma (L) and 11 (8.5%) by multiple myeloma (MM). In 82 (62.5%) patients a donor was identified at a median time from activation of 12 months (1 - 20) as of April 2003, 69 (52.5%) patients were transplanted. Characteristics (transplanted patients). Male/female ratio was 42/27. Median age 35.5 years (15 - 59). Forty-three (62.5%) patients were affected by AL, 16 (23.5%) by CML, 5 (7.0%) by L and 5 (7.0%) by MM. Early transplant was performed in 22 (32.0%) patients, while late transplant was performed in 47 (68.0%) patients. Conventional conditioning regimen (with TBI and cyclophosphamide) were employed in 62 (90.0%) and reduce intensity conditioning in 7 (10.0%). Results (Entire population). OS of the 131 patients were 42.5% at a median time from activation of 18 (3 - 76) months. OS of the transplanted patients vs not transplanted ones were 52.5% and 27.5% (p value =.05). OS of AL transplanted vs non transplanted were 59.5% and 22.0% at a median time from registration of 12 months (3.5-29) and 5 months (1-47) respectively (p value=0.027); while no difference in OS was observed for CML patients (transplanted vs not transplanted: 88.0% and 77.5% -
p value = .41). Results (Transplanted patients). Thirty-nine (56.5%) of the transplanted patients developed an aGVHD, as by April 2003, 32 (46.5%) of the transplanted patients are alive and disease-free, 3 (4.5%) are alive with disease, 9 (13.0%) died for disease and 25 (36.0%) died for transplant-related complications. OS of early vs late transplants was 75.0% and 17.5% respectively (p value = .017). Conclusions. In our experience, the probability of identifying a donor was higher than 60% at a median time from search activation of about 4 months. The OS of the transplanted patients was higher than that of non-transplanted ones (p value = .05). Moreover, transplants performed early in the course of the disease gives an advance in term of OS.

PO238
VACOP-B VS VACOP-B + HIGH-DOSE SEQUENTIAL THERAPY FOR AGGRESSIVE NON-HODGKIN’S LYMPHOMA. FINAL ANALYSIS OF THE NHLCSG
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This multi-centre randomised study compared conventional therapy (CT) with CT plus HDS and autologous PBPC rescue (ASCT) as front-line treatment for advanced-stage, intermediate- and high-grade NHL. Aims: 1) to confirm data previously reported with the use of HDS in aggressive B-NHL: CT vs CT plus HDS as salvage treatment in cases of persistent disease (PR,NR) (Arm A), and 117 pts to VACOP-B for 8 weeks plus HDS as salvage treatment in cases of persistent disease (PR,NR) (Arm B). There was no significant difference in terms of complete remissions (CR) in the two groups of pts: 67% for Arm A and 68% for Arm B. With a median survival observation time of 37 months there was no difference in 6-year probability of survival (60% and 58%), of disease-free survival (DFS) (54.5% and 65.5% for Arm A and B (p=0.7), respectively) and of progression-free survival (PFS) (41.3% and 49.5% for Arm A and B (p=0.9), respectively). Stratification of pts according to the IPI adjusted for age < 60 years showed a statistical better outcome in terms of survival (p=0.0000) and of PFS (p=0.0001) for lower-risk pts (0-1 neg factors) versus higher-risk pts (2-3 neg factors), without any statistical difference according to the treatment received. Patients with BM involvement or with T-cell NHL showed the poorest results. Retrospective analysis only including the categories of pts reported in previous randomised studies showed an improvement of our results, in line with data published before. Two pts died in Arm A (2%) and 3 in Arm B (3%) because of procedure, respectively. In conclusion, this study confirms that HDS data only in a selected group of pts with large B-cell NHL without BM involvement. There is no difference in using HDS plus ASCT after CT in all cases or only in the case of persistent disease, even in higher-risk pts.

PO239
HIGH DOSE IDARUBICIN AND BUSULPHAN AS CONDITIONING REGIMEN TO AUTOLOGOUS STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA
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Relapse still occurs in 30-50% of patients with acute myeloid leukemia (AML) following autologous stem cell transplantation (ASCT). One possibility of reducing the relapse rate is to investigate new, more effective conditioning regimens. On this basis, we developed an original conditioning program, named IBu, consisting of the combination of high dose idarubicin, given at 20mg/sqm as 3 days continuous infusion from day -13 to -11 and busulphan at 4mg/kg from day -5 to -2. As compared to the classical BuCy, cyclophosphamide, which has not a definite role in AML, was substituted with idarubicin, a powerful antileukemic agent. Here we report our experience on a series of 43 AML patients autografted in first or subsequent complete remission (CR) and conditioned with IBu regimen. There were 25 males and 18 females with a median age of 50 years (16-71). Thirty-nine patients had non M3-AML (karyotype: normal in 29 patients, unfavourable in 8 patients, no mitoses in the remaining 2 cases) in first (n=36) or second (n=3) CR, while 4 had M3-AML with t(15,17) in second (n=3) and fourth (n=1) hematologic and molecular remission. All transplants were performed using peripheral blood stem cells (PBSC) collected after consolidation treatment plus G-CSF. The median interval between diagnosis or relapse and ASCT was 4 months (2-8). The median number of CD34+ cells

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infused was 6.2x10^6/kg (1.1-16). In all patients left ventricular ejection fraction (LVEF) was evaluated before and after ASCT. Finally, for patients aged more than 60 years (n=7), both idarubicin and busulphan were reduced by one day. The median number of days with granulocytes <500/cmm and of platelets <20000/cmm was 10 (7-21) and 13 (8-95), respectively. One patient did never achieve platelets >20000/cmm and relapsed 12 months after ASCT. The median number of platelet and blood units transfused was 3 (1-7) and 3 (0-14), respectively. Extra-hematological toxicity mainly consisted of grade WHO III-IV stomatitis (40/43 or 93%), while 2 patients had grade III hepatic toxicity and one experienced transient hallucinations on day -2. Furthermore, 41 patients had FUO, while two experienced fungal infection. No transplant related death occurred. LVEF examination post-ASCT did not reveal cardiac toxicity in any patient. Finally, median time of hospitalization was 29 days (22-67). After a median follow up of 12 months (1-47), 30 patients are in continuous CR, while 13 have relapsed. Among these, 12 patients died from progressive disease, while one achieved CR2 by salvage treatment.

**PO240**

REDUCED-INTENSITY CONDITIONING WITH LOW DOSE ATG OR ALEMTUZUMAB FOLLOWED BY ALLOGENEIC PBSC: A SALVAGE THERAPY WITH AN ENCOURAGING RESPONSE RATE AND LIMITED TOXICITY IN RELAPSED/REFRACTORY MYELOMA PATIENTS


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Allogeneic transplantation (HSCT) with conventional-intensity conditioning regimens has a 50 to 70% incidence of acute GVHD, and 30 to 50% treatment-related mortality (TRM) when used to rescue relapsed multiple myeloma (MM) patients. In order to decrease the TRM, and to investigate the postulated graft-vs-myeloma effect, we employed a reduced-intensity conditioning followed by a transplant of partially T-depleted lenogastrim-mobilized hematopoietic cells. So far 15 patients entered the program: 14 myelomas and 1 plasma cell leukemia. Before allografting, 6 patients were in PR, and 9 had refractory disease. Overall, 12 failed one or more autologous transplants. Pre-transplant cytoreduction included high-dose melphalan in 6 patients and DT-PACE or thalidomide courses in the remaining 9. The median age was 57 years (range, 48-69). Conditioning regimen consisted of thiotepa 5 mg/kg, fludarabine 60 mg/m^2, cyclophosphamide 60 mg/kg, and antithymocyte globulin 7.5 mg/kg (n=9) or alemtuzumab (mabcampath) 30 mg (n=6). Donors were HLA-matched (n=14) or one-antigen mismatched siblings (n=1). GVHD prophylaxis consisted of low-dose cyclosporine and short course methotrexate. We report the results on 14 pts having a minimum follow-up of 100 days. All patients engrafted. Median time to reach 500 neutrophils and 20.000 platelets were 12 and 13 days, respectively. Acute GVHD occurred in 4 of 14 pts and was scored grade I-II in 3, and grade III in one. Chronic GVHD developed in 4 of 13 evaluable pts. One patient died of non-relapse mortality (acute GVHD and pneumonia), and 3 because of disease progression (1 plasma cell leukemia). After transplant 2 pts reached CR, 5 PR, and 7 had stable or progressive disease. Four patients received DLI, and 2 withdrew cyclosporin because of disease progression. At a median follow-up of 516 days, 10 of 14 patients are alive and disease response after immunomodulation is the following: 7 CRs (1 molecular remission), 3 PRs, 2 stable and 2 progressive disease. Although preliminary, our results show an encouraging response rate in relapsed/refractory pts. It has to be pointed out that some patients attained...
remission only after cyclosporine withdrawal and/or DLI. This latter finding further support the existence of a graft-versus-myeloma effect. Despite the relatively short follow-up and the limited number of pts, nonrelapse mortality seems rather low (7%). These results compare favourably with the outcome of conventional transplants, and show that a partial in vivo T-cell depletion can limit the incidence of acute GVHD.

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PO242
ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) FOR MYELOID LEUKEMIA (2ARY AML). RETROSPECTIVE STUDY OF 32 PATIENTS FROM A SINGLE INSTITUTION

From March 1995 to January 2003, 32 patients (pts) affected by high-risk Myelodysplastic Syndromes (MDS) or secondary acute myeloid leukemia (secondary AM L), underwent Allogeneic Stem Cell Transplantation (SCT) from HLA-identical siblings or Voluntary Unrelated Donors (VUD) in our Institution. 2ary AML was diagnosed on the basis of: a) a previous history (at least 3 months) of MDS, or b) previous chemotherapy or radiotherapy, or c) high risk cytogenetic abnormalities (i.e: chromosomal alterations involving chromosome 5, or 7, or 3q21and 3q26, or 3 or more alterations). The clinical and hematologic features of the pts were: 22 males, 10 females, median age: 40 (16-61) yrs; diagnosis: MDS: 10 pts; 2ary AM L: 22 pts; interval from 1st diagnosis to SCT: 8.5 (2-39) months. SCT was performed in 1st complete remission (RC) in 6 pts, and in 2nd RC in 1 case, while 16 subjects were transplanted in hematologic relapse or with refractory disease. The remaining 9 pts received SCT as 1st line treatment. The donors (15 males, 17 females, median age: 39.5, range 20-70 yrs) were HLA-identical siblings in 21 cases, VUD in 11, the source of hemopoietic stem cells was the bone marrow (BM) in 18 cases, and the peripheral blood (PB) in 14. ABO compatibility was present in 19 cases. The conditioning regimen included Total Body Irradiation (TBI)
in 8 pts, and was busulphan-based in the other 24 subjects. In 6 pts a Reduced Intensity Conditioning Regimen (RIC) was administered. Cyclosporin A (Cs-A) and Methotrexate (Mtx) were given to all pts as GVHD prophylaxis, and antithymocyte globulin (ATG) (Fresenius) (15 mg/kg) was added in VUD transplants. In 16 patient acute GVHD was diagnosed (grade I: 5 pts; grade II: 2 pts; grade III: 7 pts; grade IV: 2 pts) and, among 24 evaluable pts, 12 subjects developed chronic GVHD, extended in 5 pts, limited in the remaining 7 cases. Seven out of 32 pts (21.8%) died because of relapse. Overall survival of our series was 45% at 2 years, 22.6% at 4 years, and 16.9% at 5 years. Taking into account the unfavourable prognostic features of our patients, allogeneic SCT seems to be a worthwhile therapeutic tool in high risk MDS and secondary AML.

PO243
ALLOGENEIC STEM CELL TRANSPLANTATION FOR LOW-INTERMEDIATE RISK MYELODYSPLASIA: A SINGLE INSTITUTION REPORT
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Myelodysplastic syndromes (MDS) include a heterogeneous group of acquired clonal stem cell disorders characterized by ineffective hematopoiesis leading to peripheral blood cytopenias. These diseases share a predisposition to evolve into acute leukemia. Allogeneic stem cell transplantation (SCT) is the only treatment modality that has consistently been demonstrated to cure patients with MDS. However, this procedure is usually employed in high-risk MDS. We describe here our experience in 4 transfusion-dependent patients suffering from low (n. 2) or intermediate-1 (n. 2) risk MDS, according to IPSS score, who underwent conventional allogeneic SCT. According to WHO classification, two patients had refractory anemia (RA) with tri-lineage myelodysplasia, one had 5q-syndrome and one RA with blast excess, type 1 (RAEB-1). Two patients were male and two were females. The patients were 21, 22, 49 and 56-year-old at allogeneic SCT, respectively. The patient with RAEB received mini-ICE chemotherapy before transplant. Time from diagnosis ranged from 5 to 91 months. The long time to transplant (91 months) in the 5q-syndrome was due to the fact that this patient became transfusion-dependent only after 7 years of disease. Conditioning regimen consisted of fractionated TBI (12 Gy) plus cyclofosfamide in 3 patients aged less than 50 and BuCy2 for the remaining patient. In all cases unmanipulated allogeneic stem cells from HLA-matched identical siblings were used. Bone marrow was the source of stem cells for one patient (donor age < 18), while G-CSF mobilized peripheral blood stem cells were employed in the other 3 cases. Graft versus Host Disease (GvHD) prophylaxis was carried out using cyclosporin-A and short courses methotrexate. All patients engrafted, achieving a leukocyte count > 500/mm³ after a median of 15 days (range 14-22) and a platelet count > 20,000 mm³ after a median of 15 days (range 14-20). No patient showed acute GvHD, one patient developed chronic GvHD. All patients are alive and well in continue, complete remission (including cytogenetic remission, for the patient with 5q-syndrome) at +6, +12, +16 and +20 months, respectively. In conclusion, in this small experience allogeneic SCT for low-intermediate risk MDS was a feasible and well tolerated therapeutic approach, leading to the possibility of prolonged event-free survival and offering a significant perspective of a definitive cure for patients whose quality of life dramatically improved after this procedure.

PO244
REDUCED-INTENSITY CONDITIONING FOLLOWED BY ALLOGENEIC TRANSPLANTATION CAN PRODUCE DURABLE CLINICAL AND MOLECULAR REMISSIONS IN RELAPSED NON-HODGKIN LYMPHOMAS
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It has been shown that reduced-intensity conditioning (RIC) followed by allogeneic HSCT can give stable engraftment, low non-relapse mortality rate and clinical remissions in hematologic malignancies. In addition, RIC followed by allograft is feasible with a relatively low non-relapse mortality also in patients who had failed previous autologous transplantation. Herein is an update of our experience in 73 NHL and Hodgkin disease: 28 low-grade (LGNHL) (14 CLL/SL, 12 FCL, 1 MALT, 1 lymphoplasmocitoid), 30 high-grade (HGNHL) (10 T-cell lymphomas, 15 B-cell DLCL, 3 transformed, 2 rare histology), 7 mantle (MCL), 8 Hodgkin disease (HD). Median age was 49 years (range: 20-67). Before transplant, 52% had chemosensitive disease. The median time from diagnosis to allograft was 28 months (range: 5-140 months). The median number of previous chemotherapy regimens was 2 (range 1-5); 43% of pts had already failed a previous autograft. After conditioning with thiopeta (10 mg/kg), fludarabine (60 mg/ms) and cyclophosphamide (60 mg/kg), pts
PO245
LONG-TERM SURVIVAL IN CHILDHOOD WITH HIGH RISK MYELODISPLASTIC SYNDROME TREATED WITH UNRELATED CORD BLOOD TRANSPLANTATION
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Myelodysplasia (MDS) in childhood usually runs an aggressive course with a great proportion of patients succumbing in the first 2 years after diagnosis. Available evidences indicate that hematopoietic stem cell transplantation currently represents the only curative strategy for myelodysplastic children. The search of unrelated HLA-matched stem cells donors should be mandatory in MDS who lack an HLA-identical sibling. UCB may represent an alternative source of hematopoietic stem cells which may be successfully used for unrelated transplantation, primarily in pediatric patients, showing a probability of survival comparable to that obtained with other source of stem cells, as unmanipulated or T-cell depleted bone marrow. Five pediatric patients affected by high risk MDS according to IPSS were treated with intensified myeloablative conditioning regimen followed by unrelated HLA-mismatched cord blood transplantation (CBT). Four patients were considered at high-risk to evolve in AML according to the IPSS and one patient because of age over 2 years, hemoglobin F level greater than 10%, low platelets count, associated immunodeficiency and hemolytic anemia. Disease status at transplant were RAEB-t in 3 cases, RAEB and JMML in one patient, respectively. Median age of the recipients was 2.9 years (range 1.3 - 6.6). Median dose of nucleated cells (NC), CD34+ cells and CFU-GM infused after thawing were 5×10⁶/kg, 2.7×10⁴/kg and 2.35×10⁴/kg, respectively. All patients received a CBT from a mismatched donor for one locus in three cases and 2 loci in two cases; A and B HLA loci were confirmed by serologic testing while DRB1 region was studied by high resolution oligonucleotide typing. Four patients were prepared with a regimen of TBI or, in children < 3 years, busulfan followed by cyclophosphamide. VP16 was included at total dose of 20 mg/kg, according to the Eurocord guide lines for high risk hematopoietic malignancies. The patient with JMML who had already failed the allo-PBSC transplant from the mother using BUS+CY preparative regimen, received an original regimen consisting of Cytarabine, Fludarabine, VP16 and Thiotepe. All cases received ALG at dose of 600 U/kg on 4 consecutive days during the conditioning regimen. All patients received CSA and prednisone till day +28 after CBT, as GVHD prophylaxis. Before transplantation, two children were treated with chemotherapy: one showed persistence of blasts after 2 cycles of induction therapy and one patient failed a 1 locus mismatched allogeneic peripheral blood stem cell transplant from the mother, showing autologous reconstitution followed in few months by disease progression. All patients, therefore, received CBT as an upfront treatment and not as a post-remission consolidation. Four patients showed myeloid reconstitution, achieving PMN count > 500/mm³ at a median time of 26 days (range 23-30); one died of gram-negative sepsis on day +29 still in aplasia. A self-sustained PLTS recovery was documented in 2 cases after 34 and 40 days, respectively. Full donor chimerism was documented in 4 cases on day +28; one child with RAEB-t, achieved mixed chimerism, but died before subsequent evaluation. Two patients developed grade I, one grade II and one grade IV aGVHD (skin + liver). The JMML patient experienced limited gut cGVHD, resolved with immunosuppressive therapy. Two out of five patients are alive, disease free, with complete immunological recovery and with no evidence of extensive chronic GVHD at 66 and 60 months after transplant. Although the encouraging prolonged continuous complete remission of the two surviving patients, the early transplant related mortality remains a major problem of CBT. The timing, therefore,
of unrelated stem cell transplant as well as the intensity of the conditioning regimen remain controversial and further studies will be needed.

PO246
CMV CHORIORETINITIS AFTER T-DEPLETED BMT
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Chorioretinitis has often been observed in HIV^+ patients but rarely in recipients of T-cell depleted allogeneic BMT, who have undergone immunosuppressive conditioning regimens to prevent rejection and GvHD. Consequently in this patients post transplant immunological recovery is slow and they are vulnerable to viral and fungal infections. Here we describe 5 cases of CMV-related chorioretinitis in T-cell depleted HSC-transplant recipients. All of whom except one had been conditioned to transplant with a TBI-based protocol. All patients had undergone CMV reactivation from 30 to 60 days post-transplant. Chorioretinitis was diagnosed in 1 patient 3 months post-transplant and from 6-12 months after transplant in the other 4. Chorioretinitis was associated with pneumonia in 2 patients, that was fatal in 1. Chorioretinitis resolve completely in the patient with early diagnosis. In the patients with late diagnoses, retinal damage was associated with bilateral blindness in 1, and loss of sight in one eye in 2 cases. Marked reduction in vision was observed in the other patient. In our opinion CMV-related chorioretinitis may be greater than reported because:
- screening for CMV-related retinitis in not standard practice in BMT recipients;
- as the systemic infection is severe, disturbance in sight are often neglected;
- involvement of zone 2-3 in the peripheral ocular area delays the onset and diagnoses of ocular symptoms which evolve slowly and insidiously;
- presentation is atypical and very different to a standard hemorrhagic necrosis;
- the disease often resolve spontaneously as the immunological defence system become stronger.

Early diagnosis helps prevent the serious late complications of CMV-related chorioretinitis.

PO247
UNRELATED DONOR STEM CELL TRANSPLANTATION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES USING A NON-MYELOABLATIVE PREPARATIVE REGIMEN: A MULTICENTER STUDY
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A multicenter study was designed to determine the feasibility and toxicity of allogeneic stem cell transplant from unrelated HLA-matched donors after a non-myeloablative conditioning regimen. Seventeen patients were enrolled between August 2002 and May 2003; all of them were ineligible for conventional myeloablative regimens because of age, disease or complications of a previous therapy. The underlying disease was MM in 4 pts, NHL in 3 pts, AML in 3 pts, HDG in 4 pts, MMF in 1 pt., CM L in 1 and CLL in 1 pt. Twelve out of 17 pts. (70%) received more than 3 previous therapy courses and underwent autologous or unrelated transplants. Eleven pts. were in advanced disease (65%), four in PR (23%) and 2 pts. in second complete remission. The median age of pts. and donors was respectively 46 years (range, 17-58) and 39 (range, 22-44) years. As conditioning regimen pts. received Campath-1H 20 mg/day, Mel 30 mg/m², Fluda 30 mg/m² and TBI single fraction 200 cGy. All pts. received GVHD prophylaxis with CSA (3 mg/kg p.o.) and M M F (15 mg/kg×2 p.o.) respectively from t o +120, then to be tapered of 10%/week and from 0 to +35 days from transplant, then to be tapered of 25%/week. Five pts. received PBPCs collected from donor after mobilization with G-CSF and 12 pts. received bone marrow. In BM group median number of infused TNC and CD34-cells were respectively 3,1×10^6/kg (range, 0,58-4,1) and 2,45×10^6/kg (range, 1,44-8,4), in PBPC group 6,5×10^6/kg (range, 5,3-12,93) and 7,6×10^6/kg (range, 4,44-7,84). Tolerance to chemotherapy was excellent. VTNR/STR analyses or FISH studies (in sex mismatched patients) of sorted peripheral blood CD3+ T-cells were used to evaluate chimerism. Chimerism was tested at day +15, +30, +60, +90, +120, +180, +270, +365. Bone marrow aspirates (and biopsy when if positive marrow pretransplant) were analyzed by flow cytometry and cytogenetics on days +30, +60, +180, and then yearly. Donor's full chimerism was obtained in 8/10 of pts. after 30 days from transplant. 15/17 pts. reached >500 granulocyte/µL and 20.000 platelets/µL respectively after a median of 19 days (range, 0-37) and 15 days (range, 0-56). Acute GVHD was shown in 3 patients (II skin, III gut and II liver post DLI). None pts.
developed chronic GVHD. Two pts. received DLI for relapse in +96 and +186. After a median follow-up of 100 days (range 17-302) overall survival (OS) was 82%: 10 pts. achieved complete remission (59%), 2 pts. were in partial remission, 1 pt. in relapse. TRM was 12%: causes of death were infection (n=1) and acute GVHD + infection (2). These preliminary results show that a non-myeloablative conditioning regimen NMR in unrelated transplants is well tolerated also in pts with very advanced disease and it is associated with a low risk of TRM.

PO249
NON-TOTAL BDY IRRADIATION CONDITIONING FOR FAMILY HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE IN 18 PATIENTS
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Family haploidentical allogeneic stem cell transplantation is a curative option for patients with high risk acute leukemia. Conditioning regimens are aimed at maximal host immunosuppression to allow engraftment of CD34+ selected haploidentical hematopoietic cells; most regimens reported are single fraction TBI based. Here we report results of a non-TBI conditioning regimen experience in 18 consecutive patients in our institution. From December 1999 to April 2003 eighteen patients were transplanted with haploidentical hematopoietic cells for AML (4), AMI post MDS (7), chemotherapy-related AMI (2), advanced non-Hodgkin lymphoma and Hodgkin’s disease (4), multiple myeloma (1). Disease status at transplant was CR1 (2), CR>1 (6), refractory (10). Conditioning regimen was thiopeta 13.5 mg/kg, fludarabine 200 mg/mq, melphalan 140 mg/m², rabbit ATG 15 mg/kg; peripheral blood donor blood cells after G-CSF 16 mcg/kg stimulation were collected and CD34+ selected cells (Clinimacs) were infused at a mean dose of 8.1×10⁶/kg (range 5.7-14.4). CD3 mean dose at transplant was 0.8×10⁹/kg (range 0.2-1.5). No GvHD prophylaxis was administered after transplant. Two patients died before engraftment at day 2 and 17 following peritonitis and pneumonia. Sixteen out of 18 patients engratfed with ANC >500/mcl at a median time of 12 days (range 8-23) and platelets >20.000/mcl at day 18 (12-30). No cases of GvHD was observed in absence of donor lymphocyte infusion. CD3+ cell immune reconstitution was not registered in absence of donor lymphocytes add-backs in this series. Overall survival, transplant-related mortality and relapse rates in patients in remission of disease at transplant were 4/8, 3/8, 1/8 respectively; figures of patients transplanted with refractory disease were 0/10, 7/10 and 3/10 respectively, with a mean follow-up of 194 days. In conclusion, a non TBI-based conditioning regimen effectively allows engraftment of haploidentical allogeneic hematopoietic stem cells. Strategies to improve immune reconstitution and reduce late infections are under investigation.

PO250
BAVC REGIMEN AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN ACUTE PROMYELOCYTIC LEUKEMIA IN II MOLECULAR CR. UPDATED RESULTS
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In 1997 our group published an experience on 15 APL patients autografted in IInd CR, suggesting that ABMT with PML-RARα negative stem cells is likely to result in prolonged clinical and molecular remissions, whereas patients who test PCR+ at the time of harvest and transplant necessitate the use of alternative aggressive approaches. Aim of our study was to confirm these preliminary data, updating the results on the 17 patients transplanted in II nd CR with PCR negative stem cells. Patients' median age was 37 years (range 9-61) and 5 were males. The median duration of first CR was 14.5 months (range 8-23), and induction therapy of relapse included an ATRA containing regimen in all patients but one. Eleven out of 17 patients had been treated in hematologic relapse while 6 in molecular relapse. BAVC schedule was employed as conditioning regimen in all patients, followed by unpurged marrow stem cells in 16/17, while 1 patient received peripheral blood stem cells. The median interval between II nd CR and reinfusion was 3 months (range 1-6). Seven out of 17 patients relapsed after a median of 5 months from ABMT (3-12); six of them died from disease progression, while 1 patient is currently alive in her IIIrd CR after AlloBMT. Two patients presented a post-ABMT myelodysplasia testing PCR negative for PML-RARα transcript: one of them died due to secondary leukemia, and the other is alive in CR post AlloBMT. Eight patients are in hematologic and molecular CCR with a median follow up of 102 months (26-129) from transplant. Overall 10/17 patients are alive with a median follow up of 95 months from transplant (26-129). Ten-year projected probability of survival is 57% and the median has not been reached after ten years. No statistically significant difference in survival was observed neither evaluating the role of 1st CR duration (=<12 months vs >12 months), nor comparing the two different kinds of relapse (hematologic vs molecular) (63% vs 41% p=n.s.). In conclusion, ABMT still seems to be an inter-
est approach for patients with acute promyelocytic leukemia in second molecular CR, giving a chance of long-term DFS. In our series there not seems to be an advantage in treating patients at the time of molecular relapse, but these data need further evaluation on a larger number of patients.

P0251
RITUXIMAB-SUPPLEMENTED HIGH-DOSE SEQUENTIAL CHEMOTHERAPY WITH AUTOGRAFTING: AN EFFECTIVE APPROACH FOR DIFFUSE LARGE CELL LYMPHOMA (DLCL) PATIENTS WITH BONE MARROW INVOLVEMENT

Bone marrow involvement is considered as a powerful indicator of poor prognosis in DLCL. In order to improve the outcome of young patients with this adverse prognostic feature we decided to perform a pilot trial based on a Rituximab supplemented high-dose sequential chemotherapy program with peripheral blood progenitor cell autografting (R-HDS). Eligibility criteria include: i. biopsy-proven DLCL with CD20+ phenotype with no previous cytotoxic treatments; ii. bone marrow DLCL involvement; iii. age between 16-65 y.o.; iv. advanced stage disease with 2-3 aalPI score. The R-HDS regimen employed includes an initial debulking with 3 APO courses, and then sequentially: i. cyclophosphamide 7gr/m² day 1 + Rituximab 375 mg/m² (day +2 and +10), followed by PBPC harvest; ii. Ara-C 2gr/m² b.i.d. for 6 days, given with autologous PBPC support (2×10⁶ CD34+ cells/kg) and then Rituximab 375mg/m² (day +8 and day +18) followed by a second PBPC collection; iii. etoposide 2.4gr/m² day +1 + Cisplatin 100 mg/m² day +2; iv. final myeloablative regimen (M ifoxantrone 60mg/m² + L-Pam 180mg/m²), with PBPC autografting (5×10⁶ CD34+cells/kg) and then Rituximab 375 mg/sm (day +30 and +37); v. involved-field radiotherapy in areas of prior bulky lesions or residual lesions, within 2-3 mos. after autografting. Presently, 14 patients with these clinical features have been treated in five different Centers and are currently evaluable. Their median age was 50 yrs. (range: 35-65). Eight presented with disease-related symptoms and 11 had two or more involved extranodal sites. There were 1 early toxic deaths due to gram-ve sepsis following Ara-C. All the other patients collected adequate amounts of PBPC and completed their planned treatment. Overall 12 patients (85.7%) reached CR. So far, 11 (78.6%) patients are alive and 9 (64.3%) are in CCR.

With a median follow-up of 2 years, 2.5-year OS and EFS projections are 75% and 57%, respectively. In conclusion, R-HDS is feasible in this highly selected patient populations characterized by multiple poor prognostic features. Despite the short follow-up, these results indicate that R-HDS represent one of the most effective treatments in this peculiar clinical setting. Evaluation of a larger sample of patients is required to confirm the promising results of this pilot study.

P0252
OUTPATIENT MANAGEMENT IS FEASIBLE AND SAFE IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION
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Autologous stem cell transplantation (ASCT) is commonly used as treatment for patients with multiple myeloma (MM), resulting in increasing pressure on available beds. These considerations, along with the relatively low toxicity of high dose melphalan followed by peripheral blood stem cell infusion, led us to develop a program exploring the feasibility of ASCT on a mixed in/outpatient basis. Here we report our experience on a series of 26 patients programmed to be autografted according to the above model. From January 2001 a program based on early discharge at home of MM patients soon after receiving infusion of stem cells and management of complications on outpatient basis was started. Febrile neutropenia (>38.5°C and PMN<500/cm³), any WHO 3-4 toxicity and patient’s or family’s inability to cope at home were established as criteria for re-hospitalization. Accordingly, this program was offered to 26 out of 29 MM patients undergoing ASCT and all accepted it. There were 18 males and 8 females with a median age of 57 years (35-72). Before ASCT 6 patients (23%) were in complete remission, 20 (77%) in partial remission, as assessed by EBMT criteria. Median interval between diagnosis and ASCT was 6 months (range 4-60). Patients were conditioned with melphalan at 200 mg/m² in 18 cases, melphalan 140 mg/m² in 7 cases (age >60 years), and BEAM in 1 case, with a median CD34+ cells infused of 8.2×10⁶ (1.8-25.5). G-CSF was given at onset of severe neutropenia (PMN < 500/cmm). All patients (100%) were discharged on day after stem cell infusion. Readmission was necessary in 11 cases (42%), mainly for febrile neutropenia (n=5), mucositis needing total parenteral nutrition (n=5) and patient’s anxiety (n=1). Hematologic recovery was not significantly different between patients readmitted and non-readmitted (days to PMN>500/cmm and Plt>20000/cmm being 12 (11-15) vs 12 (9-13) and 11 (0-22) vs 11 (0-13), respective-
Platelet transfusion is an important tool for the prevention and treatment of bleedings in patients with hematological disorders. Indication for platelet transfusion in a prophylactic setting remain controversial and often is based on arbitrary numerical criteria. In the 1987, the National Institute of Health (NIH) identified a threshold of 20×10^9 platelets/L for prophylactic use of platelet transfusion. The appropriateness of reducing the trigger set from 20×10^9 to 10×10^9 platelets/L, in stable onco-hematological recipients, is supported by numerous studies recently published (Rebulla P et al., 1997; Wandt H et al., 1998; Lawrence J B et al., 2001, Zumberg M S et al., 2002). Interestingly, same of these studies consider the threshold of 10×10^9 inadequate in patients with febrile episode for the rapid platelet consumption observed in this condition. Based on the evidence collected in these reports and considering that the fever is a frequent complication of adult patients undergone to autologous stem cell transplantation (ASCT), we evaluated a threshold of 10×10^9 platelets/L for prophylactic platelet transfusions in these cases. In particular, we performed a non-randomized prospective analysis of the frequency of bleeding events in transplanted patients with fever, comparing the results obtained with that observed in the same patient setting with a previous platelet target of 20×10^9/L. In the first 60 patients (group A) we used a 20×10^9/L platelet trigger for administering prophylactic platelet transfusion whereas in the subsequent 75 patients (group B) we reduced the platelet threshold to 10×10^9/L. One-hundred and ten out of 135 patients showed febrile episodes (81%). The patients were examined daily for evidence of hemorrhage. Bleeding complications were defined in two main categories based on the World Health Organization (WHO) classification. Briefly, as major bleeding we defined melena, hematemesis, macrohematuria, hemoptysis, epistaxis, intracranial hemorrhage, vaginal bleeding and retinal hemorrhage with impairment of vision, while as minor bleeding mucocutaneous hemorrhages or hematomas and retinal hemorrhage without impaired vision. Overall, 61 bleeding episodes were recorded, of which 29 in the group A and 32 in the group B. Minor bleedings were recorded in 21 and 24 patients, in the group A and B respectively. Major bleeding complications were seen exclusively in 16 patients (14%), in particular there was no statistically significant difference in the frequency observed within the two subgroup of patients (7 and 9 episodes respectively). The number of platelet units transfused was reduced comparing the two arbitrary platelet trigger used (< 10,000 and < 20,000×10^9/L), in fact the ratio of platelet transfusion/patients was 2 and 1.8 respectively. In summary, our study indicate that a threshold of 10×10^9/L for platelet transfusions is safe in patients undergoing autologous stem cell transplantation as well in presence of fever with a significant reduction in platelet usage and hospital costs.
(ARA-C) + G-CSF, respectively; both courses were followed by Rituximab as purging in vivo. PBSCT with BEAM conditioning regimen by infusing the purest collection of CD34 cells was planned as last phase. Values consist at each step of therapy of bone and marrow biopsy with detection of residual disease by immune phenotype of cells, molecular biology of marrow sample when the data was already available at the beginning of therapy and detection of purging efficiency with the same methods in the product of collection after mobilization of CD34 cells; a further value was done at the follow-up. Up to May 03 36 patients have been completed the entire program; 12 have been previously treated and relapsed following the previous treatment, 24 patients did the program as up-front therapy. Histology included follicular center cell lymphoma (FCCL) in 14 pts, diffuse centroblastic lymphoma (DCBCL) in 2 pts, lymphoplasmacytic lymphoma (LPL) in 7 pts, mantle cell lymphoma (MCL) in 5 pts and chronic lymphocytic leukemia (CLL) in 8 pts. Twenty pts had leukemic manifestation. Following CHOP as first phase, all patients had a residual disease detected by immune phenotype or molecular biology. Following the first mobilization by CTX and first Rituximab 18 pts (50%) obtained a CR with absence of minimal residual disease. Following the second mobilization with ARA-C and second Rituximab 32 pts obtained a CR. Following the last phase with PBSCT 35 pts obtained CR. None of patients died for causes related to procedure, however one pt had cerebral hemorrhage and one fulminant hepatitis, 5 and 8 months after the end of program, respectively. Molecular remission was obtained in 85% of valuable pts. 27 pts remain in CR at a follow-up ranging from 5 to 47 months, mean 21 months. Results according to histology revealed a clinical and molecular remission in 93% of FCCL, a clinical and molecular CR of 60% and 20%, respectively, of CLL, a clinical remission of 40% of LPL, a clinical and molecular remission of 100% of MCL and a clinical remission of 100% of CBL. In conclusion this ongoing study shows that sequential purging in vivo therapy with the combination of rituximab and high dose therapy followed by PBSCT is highly effective in inducing CR in most of chronic lymphoproliferative disorders, however it seems that the efficacy is best expressed in FCCL and MCL.

Fungal infections caused by Aspergillus are a frequent cause of transplant related mortality. For this reason patients with severe fungal infection are usually excluded from conventional allograft transplantation. Recently, several authors suggested a role for non-myeloablative hematopoietic stem cell transplantation (HSCT) in this subset of patients. A 37-year old woman with a newly diagnosed acute myeloid leukemia (AML) FAB M1 in CR after the induction chemotherapy, during the admission for the second consolidation course, showed hepatic lesions due to aspergillus as demonstrated by hepatic biopsy. Amphotericin B liposomal complex (ABLC) at dose of 3 mg/kg/day was started. After 21 days of treatment, in reason of a significant increase in the number and volume of hepatic lesions, new anti-fungal approaches were used. Initially, Caspofungin 50 mg/day alone (for 50 days) and subsequently in association with ABLC (for other 70 days). This approach allowed a fairly good control of clinical symptoms, unfortunately without any significant modification of hepatic lesions. After eight months in concomitance with leukemia relapse a massive increase of hepatic lesions occurred. A reinduction therapy with a concomitant ABLC treatment was started again obtaining a second CR without significant modification of hepatic lesions. Since her sister was found to be HLA-A,-B, -C, -DR, -DP matched and considering the high risk of relapse and the persistence of hepatic aspergillosis, we proposed her a non-myeloablative HSCT. She received the infusion of unmodified G-CSF mobilized peripheral blood stem cells (7.9 × 10^6/kg CD34+ cells) after a conditioning regimen with Thiopeta 10 mg/kg and Cyclophosphamide 100 mg/kg. Prior and during the transplantation she received therapy with ABLC (3 mg/kg/day), anti Herpes Simplex Virus prophylaxis with acyclovir and anti Pneumocistis Carini prophylaxis with Trimetoprim-sulphamethoxazol. Engraftment was prompt with an ANC of > 500/µL on day 14 and platelet > 20.000/µmL on day 11. Chimerism studies made on day 30 post-transplant using polymorphic STR genes showed 100% of marrow cells, B and T cells of donor origin. An abdomen CT scan performed on day 28 post-transplant showed a dramatic resolution of hepatic lesion. In reason of the residual hepatic lesions, from day 32 until day 81 post-transplant the patient received therapy with voriconazole (200 mg/day). The patient developed grade 2 cGVHD primarily affecting the skin and completely resolved with steroid therapy associated to extracorporeal photopheresis. The immune reconstitution after HSCT was fast with CD4+ T cell subset never below the value of 400/µL. Actually, the patient is alive and well after a follow up of 1 year without cytological evidence of disease and with stable donor chimerism (95-100%). The last CT scan performed (1 year post-transplant) showed a complete resolution of hepatic nodules. In conclusion, the reduced toxicity, the prompt engraftment and the faster immune reconstitution after non myeloablative HSCT.
allow an effective treatment for high risk leukemic patients with concomitant aspergillosis ineligible for conventional allografts.

PO256
AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PRIMARY REFRACTORY OR RELAPSING HODGKIN’S DISEASE: COMPARISON BETWEEN CD34+ IMMUNOSELECTED AND UNSELECTED STEM CELLS GRAFT
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Autologous peripheral blood stem cell transplantation is well accepted in treatment of high risk Hodgkin’s lymphoma. Some authors described the presence of Hodgkin cells in peripheral stem cells collections although the correlation between the presence of tumor cells in the grafts and the incidence of relapse after high-dose therapy is not well understood. The positive selection of CD34+ cells from PBSC has been demonstrated an efficient purging method reducing the number of tumour cells in the graft. The role of CD34+ selection on the clinical outcome of Hodgkin disease is still controversial. We report the results of hematological reconstitution, clinical outcomes, incidence of infection, disease free and overall survival in a group of 11 patients affected by refractory or relapsing Hodgkin disease receiving CD34+ selected PBSC (group A). These patients were compared to a group of 11 patients receiving unmanipulated PBSC for refractory or relapsing Hodgkin disease (group B). Patients in the two groups were matched for response to conventional chemotherapy and for disease state at transplantation. Median age was 23 years (range 17-42) in A group and 30 years in B group (range 15-35) (p=ns); four patients were male and seven female in group A, while six patients were male and five female in group B. The median IPI score was 2 in both groups. Disease stage at the diagnosis was III-IV in the majority of patients. B symptoms were present in more than 80% of patients. In both groups histologic findings consisted of sclero-nodular subtype of Hodgkin’s lymphoma in 8 out of 11 patients. All patients received a conventional first-line polichemotherapy regimen as ABVD, BEACOPP, STANFORD V; response to treatment was: CR in 3 pts (27%), PR in 2 pts (18%) and PD in 6 pts (55%) in both. Median time to relapse for patients achieving CR was 25 months. Relapsing patients and primary refractory patients, were submitted to salvage chemotherapy with MICA (Mitoxantrone, Carboplatinum, Methylprednisolone and Aracytin) and stem cell harvest from peripheral blood and were then sched-uled for HDT. In group A stem cells were submitted to positive immunoselection using the Ceprate SC system (Cellpro, Bothell, WA, USA) or the CliniMACS device (Miltenyi Biotech GmbH, Bergish-Gladbach, Germany). Patients received a median number of 4.74×10⁶/kg and 8.45×10⁶/kg CD34+ cells respectively in A and B groups (p=0.23). No difference was observed between the two groups regarding the hematological engraftment, clinical outcomes, incidence of infection, disease free and overall survival. Two years after transplant the OS and DFS rates were 73% and 57% in A group and 82% and 100% in group B respectively. These results show no advantages for patients receiving autologous immunoselected CD34+ stem cell transplant compared to unselected PBPC transplant. CD34+ selection is a procedure requiring substantial human and financial resources but its use in relapsing or refractory Hodgkin’s disease is not supported by our data.
PO257

IMATINIB ASSOCIATED WITH PEGYLATED INTERFERON IN THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA IN EARLY CHRONIC PHASE: RESULTS OF A PHASE I/II STUDY


The Italian Cooperative Study Group on chronic myelogenous leukemia (CMML) has conducted a phase I/II study aimed to evaluate the safety (primary endpoint) and the efficacy (secondary endpoint) of a combination of imatinib (400 mg daily) and pegylated interferon (3 subsequent cohorts at 50, 100, 150 micrograms weekly) in early chronic phase, previously untreated Ph positive CML. Seventy-six patients have been enrolled by 18 centres of the ICSG on CML from July to December 2001. Twenty-seven patients have been enrolled in the first cohort, 18 in the second and 31 in the third cohort. Forty-four were males and 32 females, the mean age was 47 yrs (18-68 yrs); 34 (44%) were low Sokal risk, 24 (33%) intermediate and 18 (23%) high risk. The present analysis has been done when all the patients have been followed for a minimum of 12 months (core phase of the trial). Safety: overall, 44/76 (58%) discontinued permanently PegIFN, 22/44 (29%) for hematopoietic adverse events (AEs) and 22/44 (29%) for extra-hematologic AEs. The proportion of patients who discontinued permanently PegIFN within 12 months was similar among the 3 cohorts, being 52%, 61% and 61% in cohorts 1, 2 and 3, respectively. However the proportion of patients with grade III/IV hematologic AEs was lower in cohort 1 (52%) with respect to cohort 2 (83%) and 3 (61%). The same trend was observed for extra-hematologic AEs, particularly of grade III, being 22%, 33% and 55% for cohorts 1, 2 and 3, respectively (only 2 episodes of grade IV extra-hematologic AEs were recorded, both among cohort 2 patients: a anaphylactic reaction and a profound asthenia). Efficacy: 74/76 (97%) got a complete hematologic response. The rate of major cytogenetic response (M CgR) was 83% (partial CgR 13% and complete CgR 70%). The rate of M CgR was significantly lower in high Sokal risk patients (56%) as compared with intermediate (91%) and low Sokal risk patients (91%) (p=0.002); similar differences were recorded with the Euro score: 55% for high risk, 79% for intermediate and 92% for low risk pts. Conclusions and suggestions: the association of imatinib and PegIFN is feasible but at the dosages used in this study the compliance was poor. The combination is very effective, being the CrA rate significantly risk-related.

PO258

ITALIAN COOPERATIVE STUDY GROUP IN CML: STANDARDIZATION AND QUALITY CONTROL PROGRAMS OF REAL TIME RT-PCR FOR MINIMAL RESIDUAL DISEASE DETECTION OF BCR/ABL GENE TRANSCRIPT

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Assessment of therapy efficiency is a challenge in current treatment protocols for chronic myeloid leukemia and acute leukemia. The clinical impact of minimal residual disease detection by classical RT-PCR analysis of chromosomal translocations has been shown on large series in CML and acute leukemia patients. Recently, real time quantitative RT-PCR has permitted high throughput quantitative analysis. Three out of 26 European Laboratories from 10 countries have collaborated to establish a standardized protocol for RQ PCR (Taqman) for the main leukemia associated translocations within the Europe Against Cancer Program (EAC) of the EU. The network was organized as 9 fusion gene (FG) groups with 1 group for control genes. Four phases were scheduled: 1) training with experiment using cell line and minimal residual disease detection by classical RT-PCR analysis of chromosomal translocations has been shown on large series in CML and acute leukemia patients. Recently, real time quantitative RT-PCR has permitted high throughput quantitative analysis. Three out of 26 European Laboratories from 10 countries have collaborated to establish a standardized protocol for RQ PCR (Taqman) for the main leukemia associated translocations within the Europe Against Cancer Program (EAC) of the EU. The network was organized as 9 fusion gene (FG) groups with 1 group for control genes. Four phases were scheduled: 1) training with experiment using cell line and DNA samples. Reagents for PCR were mainly provided by PE Biosystems, cell line or patient RNA and DNA were distributed by each FG group leader and the plasmid dilutions were centrally produced in Marcella. During the second QC round, we preformed an
equilibrated statistical assay: 9 targets were selected (the main transcripts), laboratories were randomly chosen to perform the analysis on 5 blind RNA samples (2 neg, 10(e)-1, 10(e)-3, 10(e)-4); each participant testing 4 targets (100 plates total). Results. 1) A standardized protocol has been approved for the RT and PCR steps. 2) Several potential control genes have been evaluated leading to the selection of 3 stable expressed gene (GUS, ABL and b2 microglobulin (B2M)) based on their comparable expression levels in bone marrow and blood samples from leukaemic patients and healthy controls. 3) EAC primer and probe sets for FG have been selected with a threshold of detection of 10 molecules of plasmid and 10(e)-4 of RNA dilutions reached by all testing labs 4) 2 first QC rounds: 8.6% & 2.8% false positivity and 6.3% & 3.4% of false negativity. Conclusions: The European standardisation was achieved. Normalisation with control gene is mandatory. EAC protocol allows data comparison between laboratories. Furthermore, three reference labs (Bologna, Naples and Turin) of the Italian Cooperative Study Group on CML (ICSG-CML) apply this molecular tool to assess the BCR-ABL/B2M transcripts ratio. The aim of the study was to assess the efficiency of Imatinib therapy to induce molecular response in 200 out of 324 chronic myeloid leukemia (CML) patients entered in a phase II clinical trial of ICSG-CML. For patients with CML, methods for monitoring response to treatment have changed considerably in recent years but prediction of response to Imatinib could not be clearly anticipated by cytogenetic analysis. The quantitative QRT-PCR has proved extremely valuable for assessing and monitoring minimal residual disease in patients who achieve Ph negativity with imatinib mesylate and for predicting response to the drug. Bone marrow samples (BM) were collected before treatment, after 3 and 6 and 12 months at the end of study treatment (12 moths) while peripheral blood samples (PBL) were obtained after 6, 10, 14, 20 and 56 weeks of therapy. Results are consistent with the observation that the majority of patients obtaining a complete or major cytogenetic remission could be predicted by monitoring serially of decreasing numbers of BCR-ABL transcripts at two months after starting therapy in PBL.

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were registered in 7% and 17% of patients, respectively. A drug withdrawal occurred in 9 patients (4 compliance loss, 1 peripheral neurotoxicity, 1 protocol violation, 1 hypertransaminasemia, 1 blastic evolution, 1 thyroid cancer). The toxicity of PEG Intron was WHO grade I (leukopenia 40%, hypertransaminasemia 34%, fever 33%, alopecia 19% and peripheral neurotoxicity 10%); WHO grade II (mainly fever 33%, leukopenia 11% and hypertransaminasemia 7%); WHO grade III (1 case of leukopenia and 1 case of hypertransaminasemia). Ten patients showed significant alteration of the laboratory thyroid parameters (2 hypothyroidism and 8 hypothyroidism), requiring PEG Intron dose reduction in 3 cases and transitory discontinuation in 1 case. Neither thrombotic nor hemorrhagic events were observed during the first year of treatment. In the second year of the study (part II) the 76 responding patients, 64 with HR and 12 with Partial Response (Platelets 500–600×10^9/L), continued PEG Intron treatment at progressively decreasing dose in order to maintain the Response. Till now sixty patients completed the second year of the study, 40 (66%) with HR, 10 (17%) with Partial HR and 10 (17%) with minor HR (Platelets 600–750×10^9/L). These preliminary data show that PEG Intron at relatively low dose is able to induce and to maintain the HR in the majority of patients.

### PO261

**Splenectomy in Patients with Myelofibrosis with Myeloid Metaplasia: The Main Risks and Benefits**

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Introduction: splenectomy may contribute to improve outcome of the patients with myelofibrosis with myeloid metaplasia (MMM) refractory to therapy, but it is associated with significant perioperative mortality and morbidity. We evaluated risks benefits ratio of splenectomy in patients with MMM, with particular attention to thrombotic and hemorrhagic complications. Methods: we retrospectively analysed 14 MMM patients (8M, 6F), who underwent splenectomy between September 1996 and October 2002. The median age at splenectomy was 64 years (range 44–74 y). Median time from diagnosis of MMM to splenectomy was 25 months (range 0–104 m). The patients have been classified according to prognostic Dupriez score in 3 groups: low (4), intermediate (8) and high risk (2), respectively. Primary indications for splenectomy were: progressive transfusion-dependent anemia (11/14), symptomatic splenomegaly (9/14), severe thrombocytopenia (4/14), portal hypertension (3/14). Only one patient was electively submitted to surgery before allogeneic stem cell transplantation from HLA identical donor family. All patients were subjected to vaccine prophylaxis and 13/14 patients (92.8%) received anti-thrombotic prophylaxis with low molecular weight heparin (LMWH). Results: evaluation after 6 months revealed: improvement in constitutional symptoms related to splenomegaly (55%), reduction of transfusion dependence (63.6%), increase of platelet count (25%). The main peri- and post-operative complications included infections (8), acute bleeding (1), thrombosis (2) and extreme thrombocytopenia (5). Leukemic transformation occurred in 3 of 14 patients (21.4%). In addition to prophylactic LMWH, 5 patients with rebound thrombocytopenia (PTL>1.000×10^9/L) received ASA, Hydroxyurea was administrated only for concomitant leucocytosis (WBC>30×10^9/L). Perioperative early mortality (within 30 days) was 21.4% (3/14): all patients died for thrombotic or hemorrhagic events, particularly 2 patients for pulmonary embolism and 1 patient for...
Thrombosis represents the major cause of morbidity in patients with essential thrombocythemia (ET), although no clear correlation with platelet counts or other clinical/laboratory characteristics has been identified yet. Since ET often occurs in young subjects without other clinical risk factors, the identification of biological markers associated with thrombosis would be highly desirable in order to choose the most appropriate treatment, if any. Furthermore, the diagnosis of ET is mainly based on negative criteria (PVSG), rather than positive ones, and search for new diagnostic markers is actively pursued. Recently, the presence of clonal hemopoiesis has been correlated with a greater occurrence of thrombosis in ET if compared to patients with polyclonal hemopoiesis. The aim of this study was to evaluate clonal hemopoiesis, erythropoietin-independent erythroid colony (EEC) formation, and PRV-1 expression in patients with ET, and their association with clinical and hematologic characteristics. Forty-two women (median age 43) with a diagnosis of ET based on the PVSG criteria were studied. Clonal hemopoiesis, evaluated with the HUMARA assay, was informative in 37/42 patients (88%); of these, 20 were clonal and 17 were polyclonal, 54% and 46%, respectively. Seven patients/37 (19%) had suffered from thrombotic events at the time when diagnosis of ET was established; no further major thrombotic events were recorded in the follow-up (range, 9–72 months). Among the 20 patients with clonal hemopoiesis, six (30%) had had either, or both, major thrombosis or minor occlusive vascular events at diagnosis, as compared to 1 patient among the 17 with polyclonal hemopoiesis (6%); furthermore, this patient was found to be positive to lupus anticoagulant test on a later evaluation. EEC growth from the peripheral blood was determined using a methylcellulose assay in 28/37 patients, and found to be positive in 12 of them (43%). Although the difference did not reach the statistical significance, 53% of clonal patients in whom EEC were analyzed (6 out of 15) were EEC+, as compared to 30% (4 out of 13) of polyclonal patients. The expression of PRV-1 was determined on peripheral blood granulocyte DNA by a semiquantitative RT-PCR assay in 33/37 patients; of these, 17 were clonal and 16 were polyclonal, 54% and 46%, respectively. PRV-1 expression levels higher than controls were found in 12 out 17 clonal patients evaluated (70%) as compared to 12 out 16 (75%) polyclonal patients. There was no correlation between clonal hemopoiesis and either EEC or PRV-1, as well as between each of these and clinical characteristics, including: age, number of platelets or white blood cells at the diagnosis, splenomegaly, and hemorrhages. There was also no correlation between EEC and PRV-1 assay, as well as between these and thrombotic events. The results of this study suggest that clonal hemopoiesis as detected by the HUMARA assay might represent a risk factor for thrombosis in female patients with ET, while determination of either PRV-1 or EEC does not offer significant information under this respect.
PO264

SIGNIFICANCE OF CYTOGENETIC ABNORMALITIES ADDITIONAL TO PH IN THE RESPONSE TO IMATINIB MESILATE IN CHRONIC MYELOID LEUKEMIA PATIENTS IN CHRONIC PHASE


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Chronic myeloid leukemia (CML) is characterized by the presence of the reciprocal translocation t(9;22) (q34;q11), resulting in a BCR-ABL fusion gene. At diagnosis of CML, the frequency of abnormalities additional to Ph chromosome ranged from 7 to 10%, according to several series. The most common of them were trisomy 8 (30-35% of case), double Ph (30%), isochromosome 17 (20%), trisomy 19 (13%), loss of Y (8% of male patients), trisomy 21 (7%), trisomy 17 (5%) and monosomy 7 (5%). The clinical impact of additional cytogenetic abnormalities is most likely modified by the treatment used. The Italian Cooperative Study Group on CML (ICSG on CML) has activated a phase II multicenter observational study, aimed to evaluate the efficacy and safety of STI-571 (inhibitor of the protein tyrosine kinase bcr-abl associated) in adult patients with Ph+ CML chronic phase, failing α-IFN therapy for hematologic and cytogenetic resistance or intolerance. To asses cytogenetic pattern of patients enrolled into the study, the karyotype was performed in 300 patients. At the baseline, 25/300 patients (8.3%) showed additional aberrations in Ph+ clone. Of these, trisomy 8 was observed in 12 patients (48%). In 9 cases, +8 was the sole additional abnormality; in the remaining three patients, it was associated to double Ph, to loss of Y and involved in a more complex karyotype, respectively. The second most frequent abnormality was loss of Y, found in 5 patients (20%) and one showed a complex karyotype. Double Ph chromosome was described in 5 pts and as sole additional abnormality in 2 patients. The last five patients showed a karyotype with different cytogenetic aberrations. Each patient was studied every 3 months to evaluate hematologic and cytogenetic response (CR). Twenty-one patients (84%) reached a complete hematologic remission (CHR); 4 patients went off treatment before 1 year for unsatisfactory therapeutic effect. As regard the additional aberrations, 3 of these 4 cases carried an additional Ph chromosome. At 1 year of treatment, the major CR occurred in 12 patients: 10 achieved complete CR (40%). Eight of 9 patients with a sole +8 reached major CR, meanwhile the three pts with +8 associated to other abnormalities were cytogenetic no responders. Five patients still show the same additional abnormality observed at the baseline. In particular, in the trisomy 8 group, only one patient retained this aberration. The other 4 ones had loss of Y: 2 of them, at the screening, showed also other abnormalities. It’s also remarkable that usually loss of Y may be a costitutional marker related to age of patients. Although Ph+ CML patients with clonal evolution can reach a complete CR, the impact of additional abnormalities on clinical response can have important clinical and biological implications according to the type of cytogenetic changes.
ELDERLY PATIENTS WITH PH+ CHRONIC MYELOGENOUS LEUKEMIA (CML): RESULTS OF IMATINIB MESYLATE TREATMENT


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Thirty-two patients with Ph + CML aged more than 60 years were treated with the tyrosine-kinase inhibitor Imatinib Mesylate at our Institution. There were 17 males and 15 females, median age was 65.3 years (range 60.2-78.7); median interval from diagnosis was 44 months (range 2.8 - 159). 21 patients (Group A) were in chronic phase (CP) and resistant/intolerant to α-Interferon (IFN) (14 patients) or not eligible for IFN and previously treated with hydroxyurea (HU) (7 patients), with a median interval from diagnosis of 39.8 months (range 2.8-146); according to Sokal score, 7 patients (33%) were at low risk, 9 (43%) and 5 (24%) at intermediate and high risk, respectively. The remaining 11 patients (Group B) were treated in accelerated/blastic phase (AP/BP) of disease after a median interval from diagnosis of 46 months (range 6.3-159). In the Group A, 5 patients are too early (< 6 months of treatment), 1 patient stopped the treatment after 2 months for a severe skin toxicity and 15 patients are evaluable for response at 6 months; complete hematologic response (CHR) was achieved by all patients and complete karyotypic response (CKR), defined as 100% of Ph-negative metaphases, was obtained in 12/15 patients (80%). Toxicity was mild: 3 patients had a transient cytopenia, 2 a skin reaction and 1 muscular pain. After a median follow-up of 10.6 months (range 0.5 - 35) all 15 patients are alive in persisting response. In the Group B, 1 patient died from infective complication in aplastic phase after 3 months, 1 patient had an early progression and died after 5 months, 9/11 patients are evaluable for response at 6 months: all these 9 patients achieved CHR and 3/9 (35%) achieved also CKR. As concerns toxicity, 9/11 patients had a cytopenia, which was transient in 8/9 while 1 patient died in aplasia; skin reactions were observed in 2 patients and atrial fibrillation occurred in 1 patient. Median survival of the whole group B patients was 13.3 months: 1 patient died from toxicity, 4 patient died from progressive disease, 1 patient is alive in AP and 5 patients are still alive in CHR, 3 of them being in CKR. Present data indicate that Imatinib Mesylate is safe also in elderly patients with CML, with very low toxicity especially in CP; clinical results are at least as good as in younger patients, with a high rate of CHR in all disease phases and also of CKR in CP.

PO265 DECITABINE UP-REGULATES THE EXPRESSION OF THE CANCER-ASSOCIATE PRAME ANTIGEN IN EARLY CHRONIC PHASE CML CELLS

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A large body of clinical and experimental evidences indicate that CML cells in specific contexts are able to induce an efficient immunological reaction in the hosts. Indeed, in the AlloSCT setting, it has been shown that Ph + cells cannot always be eradicated by high doses of chemoradiation conditioning of AlloSCT, and the immunological graft-versus-leukemia reaction gives a great contribution to disease eradication. In addition, recent data on the long-term follow-up of CML patients treated by α-interferon (IFN) indicates that 13% of cases are induced into continuous CCR by the treatment and a significant proportion of these latter (46%) remains in CCR for more than 10 years despite some of the patients discontinued the IFN treatment. It is then plausible that IFN treatment may induce a long-term immune-mediated control of residual Ph + cells in those cases. In the present study, we investigate the PRAME antigen, a novel CML-specific antigen belonging to the cancer/testis associated antigens (CTAs). This tumor associated antigen is an attractive target for immune-based therapies because its expression in several tumor tissues including melanomas and renal carcinomas, and its absence from normal tissues except testis. Very recently, it has been demonstrated that PRAME antigen epitopes can be efficiently presented to cytotoxic T lymphocyte in the context of MHC-I restriction. We firstly studied, by a quantitative Real Time PCR assay, the expression of PRAME in bone marrow precursors of 30 patients with chronic phase CML; 10 of these were untreated patients at diagnosis of the disease, while the remaining had a long duration of the disease and have received previous treatments (IFN, LD Ara-C, and/or hydroxyurea). None of the latter group of patients had major (complete + partial) cytogenetic conversion at the time of inclusion in the study. PRAME gene was found to be expressed, but at low levels, in 8 of the 10 newly diagnosed patients (mean level of PRAME specific mRNA was 0,05±0,06). Interestingly, the level of expression of the PRAME gene was found to be higher in the group of late CP-CML patients who always were positive for the expression of this antigen. In addition we found a rough correlation between the level of PRAME expression and the time from the diagnosis: the mean level of expression were 0,24±0,53 and 0,55±0,99 in patients with less than 60 mo of previous treatment and in patients with...
more than 60 mo, respectively. These results suggested that during the course of the disease, the expression of PRAME gene tends to increase. To verify whether the expression of this gene is modified by the more common used drugs in CML or by methylation status at the CpG islands of DNA, we incubated the KT1, a Ph+ cell line, and Ph+ primitive cells from untreated patients in the presence of scalar amount of IFN (from 10 to 200 U/mL), of Imatinib (0.1 to 1.0 µM), and of hydroxyurea and of the demethylating agent decitabine (1 to 5 µM). Our findings indicated that none of the drugs with a known effect against the Ph+ cells was capable to modify the expression of PRAME gene, while decitabine showed a dose-dependent effect in the inducing the expression of this gene. Indeed, we found that after 48 hours of in vitro incubation of Ph+ cells in the presence of decitabine, the level of PRAME specific mRNA increases up to 15 fold respect the untreated control cultures. As expected, the wash-out of the drug resulted in a gradual decrease of the PRAME expression. Taken together, these results indicate that methylation status at specific DNA sites in involved in the expression of PRAME antigen in CML and in its gradual increase in the late CP-CML. In addition, we showed that a demethylating agent already used in the treatment of clonal hematopoietic disorders, the decitabine, is able to up-regulate PRAME gene also in early CP-CML cells, thus supporting the possible use of this drug to induce an immune-mediated control of CML.

**OTHER TARGETS FOR IMATINIB THERAPY**

More than 60 cases in which CML appeared following a neoplastic disease, including ioper-eosinophilia. Eosinophilic syndrome (HES) is a rare hematologic disorder characterized by persistent eosinophilia with organ involvement. Imatinib therapy seems to be effective in these patients. Mutations of c-KIT causing spontaneous activation of the KIT receptor kinase are associated with sporadic adult human mastocytosis (SAHM) and with human gastrointestinal stromal tumors. KIT-activating mutations such as Asp816Val mutant KIT, are supposed to be resistant to imatinib therapy while “regulatory” c-Kit mutation are most likely sensitive. After genotype of Kit mutations we treated three SAHM affected patients. We report the in vitro and effective clinical use of Glivec therapy in these diseases.

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ease treated only with surgery, or without prior therapy, have also been described in literature, while nothing is yet known about non-treatment-related CML (nTr-CML) as a second neoplasm. The characteristics of the very rare nTr-CML cases have never before been analyzed. The literature up to December 2002 was screened using the Medline database to identify cases of Tr-CML and nTr-CML. We found a total of 81 cases with secondary CML; among them, 4 (5%) were nTr-CML. Moreover, we considered 5 cases with nTr-CML identified among 270 newly diagnosed CML at our Department. Our report thus considers 9 cases with nTr-CML compared to 77 affected by Tr-CML as a secondary neoplasm. The median age at the appearance of the first tumor was compared in the two groups: it was 46 yrs in the Tr-CML group and 74 yrs in the nTr-CML group (p < 0.0001). This difference remained even when the patients in the Tr-CML group were subdivided according to previous therapy: CHT (p = 0.001), RT (p < 0.0001), and CHT plus RT (p < 0.0001). Hence, the median age at CML diagnosis was significantly higher in the nTr-CML than in the Tr-CML group (78 yrs versus 53 yrs, p < 0.0001, respectively). The median age of CML onset was confirmed to be higher in the nTr-CML patients even when they were subdivided according to the previous therapy regimen: CHT (p = 0.002), RT (p = 0.0004), and RT plus CHT (p = 0.0002). Comparison of the latency period between the nTr-CML and the Tr-CML groups revealed no important difference, being 53 mo. versus 63 mo. (p = 0.3), respectively. No difference was observed even when the Tr-CML patients were divided into the three groups according to previous therapy (CHT, RT, and CHT plus RT). No significant difference was observed between the two groups in terms of male or female gender. The proportion of hematological malignancies as first tumor type was not different in the two groups (44% in nTr-CML versus 56% in Tr-CML), although lymphoma was the most frequent first tumor among Tr-CML cases whereas there was no case in the nTr-CML group (p = 0.05). Our study underlines that nTr-CML as a second malignancy is a rare entity associated with elderly age. These data suggest that nTr-CML as a second neoplasm could be due to the effect of the immunological alterations produced by the first neoplasia, in addition to those distinctive to elderly age.

PO269
DIFFUSE LYMPH NODE INVOLVEMENT AS PRIMARY DISEASE LOCALIZATION OF EXTRAMEDULLARY MYELOID BLAST CRISIS IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS TREATED WITH IMATINIB

Nodal extramedullary myeloid blast crisis represents a rare event in CML. Diffuse lymph node involvement as unique localization of myeloid blast crisis following imatinib therapy has not been described yet. We report the clinico-biologic features of two patients developing multiple lymph node involvement as primary disease progression site, with hemogram, bone marrow and cytogenetics still consistent with a persisting chronic phase. Patient #1: A 65 year old female was diagnosed Ph+ CML in November 1997 and received hydroxyurea for 4 months (mos). From april 1998, she was given Interferon α (α-IFN) plus oral cytarabine for 1 year, obtaining a complete hematologic (CHR) and cytogenetic responses, followed by α-IFN alone for additional 2 mos. On June 1999 the patient developed a new chronic phase with 70% of Ph+ metaphases. In September 2001 (100% Ph+), she was started on Imatinib therapy (400 mg/day), obtaining a CHR at one mo and a major cytogenetic response at the third mo. Imatinib was continued for additional 15 mos. In march 2003, the patient developed fever, asthenia, myalgia and multiple bilateral lymph node enlargements (lateral-cervical, axillary and inguinal). Hemogram and bone marrow were still consistent with chronic phase while marrow cytogenetics showed 86% Ph+ metaphases without other abnormalities. A CT scan confirmed the diffuse lymphadenopathy and a PET-FDG imaging showed an elevated glucose uptake in all involved lymph nodes. No significant splenomegaly was present. An excisional biopsy of lateral-cervical lymph nodes documented a massive tissue infiltration by CD45+, CD34+, CD117+ and CD43+ blast cells. Flow cytometry and karyotypic analysis of lymph node cells confirmed the massive presence of myeloid blasts (CD34+, CD33+, CD13+, HLA-DR+, CD19 CD5−, CD7−) with a complex hypodiploid karyotype: t(9;22), iso 17, der 6 (del 6q). Treatment with fludarabine/ARA-C resulted in a complete remission with total disappearance of lymphadenopathy and negative CT and PET-FDG scans. Patient #2: A 49 year old female was diagnosed Ph+ CML in February 1995 and treated with hydroxyurea for 6 years (1995-2001) due to α-IFN intolerance. On January 2001 the patient developed an accelerated phase and was started on Imatinib (600 mg/day) obtaining a CHR and a major cytogenetic response after 8 mos. After additional 4 mos of therapy, the patient returned to chronic phase (65% Ph+) and was sequentially treated with hydroxyurea, busulfan and mercaptopurine. On February 2003, with hemogram, bone marrow and cytogenetics still indicating chronic phase, the patient developed a cervical lymph node enlargement. Needle aspiration revealed a...
myeloid blast cell population with a 46 XX t(9;22) karyotype without other abnormalities. Treatment with idarubicin, etoposide and ARA-C was unsuccessful and she expired early afterwards. These two cases, albeit of different biologic dynamics, appear of interest under several aspects. First, they represent to our knowledge the first report of nodal involvement as unique progression site in CML patients treated with imatinib. Second, it appears, at least in case n.1, that imatinib ‘pressure’ has probably selected a resistant Ph+ cell clone unable to expand in the bone marrow but with unusual homing capacity. These cells might have found a ‘permissive’ microenvironment in lymph nodes allowing their growth and expansion. Interestingly, the der 6 (del 6q) abnormality, only found in lymph node blasts, is unusual for blast phase CML but of rather frequent detection in B-cell NHL. Analysis of kinase domain mutation in lymph node blasts is ongoing. Based on this data, studies addressing imatinib-related changes in the homing capacity of Ph+ myeloid progenitors are strongly warranted.

PO270
CYTOGENETIC ABNORMAL CLONES PH'-NEGATIVE EMERGED DURING IMATINIB THERAPY SEGREGATE MAINLY IN CD34+ STEM CELLS AND PRESENT VARIABLE GROWTH IN CELL CULTURE
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Imatinib mesylate (Glivec, Novartis) is a specific inhibitor of tyrosine kinase with specific, targeted action against BCR-ABL cells. This drug has demonstrated superior activity and very high tolerability compared to other treatments for CM L. Sustained complete hematologic responses and major and complete cytogenetic responses are common. However, little is known regarding long-term outcomes. In our Institution we treated 63 CM L patients in different phases of the illness with Glivec since November 2000. In 3 patients the emergence of a cytogenetic abnormal clone in cells Ph'-negative were evidenced after a median of 23 month since the beginning of imatinib therapy. These 3 patients have all started Glivec while in chronic phase for interferon intolerance. Median age was 52 years, sex female, median time from CML diagnosis 40 months. None of the patients had ever progressed to accelerated or blastic phase. Previous treatments included hydroxyurea, 6MP, Ara-C and interferon. One patient harvested stem cell after mini-ICE therapy. Cytogenetic at onset and after starting Glivec was characterized by the presence of Ph' chromosome. No additional abnormalities were ever seen. All patients have achieved a good response to Glivec with 2 major and 1 complete cytogenetic remission when additional abnormalities were noticed in Ph'-negative cells. Two patients presented with +8 in 50 and 60% cells, one patient presented with -7 in 11% cells. Retrospective analyses of stored pellet using FISH did not evidence abnormalities in previous samples. At this time bone marrow presented with reduced cellularity, normal differential and mild dysplastic signs as documented in patients responding to Glivec. Three months later bone marrow was repeated, and the presence of the abnormal clones confirmed. Bone marrow cells were separated into CD34+ and CD34- negative, while short and long term cell culture were set up. FISH analysis on CD34+ and CD34- cells evidenced that the abnormal clone segregated into the CD34+ compartment suggesting the involvement of an early stem cell. Cell culture evidenced normal growth in two patients and dysplastic growth in one patient. FISH analysis of cultured cells did not evidence growth advantage for Ph+ or the new clone. Simultaneous FISH analysis for Ph', +8, or -7 were also performed and confirmed that new abnormalities were present only in Ph+ cells. One patient that had lost cytogenetic remission had three additional bone marrow analyses that showed that the percentage of the Ph' cells inversely correlated to the -7 clone being higher when the Ph' was reduced and vice versa (Ph'=70%, -7=7%; Ph'=46%, -7=25%; Ph'=25%, -7=35%). Although the additional abnormalities could arise from a stem cell previously damaged, our experience together with the few cases reported in the literature, suggest that Glivec could be implicated in the emergency of the new clones, whose origin and development remain to be determined. These data, although regarding a limited number of patients, suggest caution and careful monitoring of all patients treated with Glivec and recommend the registration of larger series of patients to clarify the role of the molecule and the long term clinical outcome.

PO271
EFFICACY AND SAFETY OF IMATINIB TREATMENT IN ELDERLY PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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The aging population has been increasing in recent decades. Studies on imatinib (STI) therapy for the treatment of chronic myeloid leukemia (CML) have been performed in patients < 70 years of age. The present study is aimed to evaluate the efficacy and toxicity of this drug in elderly patients with CML, in consideration of its cost. For this purpose we analysed a series of 16 patients, 71-83 years of age (mean age 75.5 years),
observed in our unit in the last 18 months. All cases fulfilled the criteria for CML diagnosis in chronic phase, Ph1+ and/or bcr/abl positive. The majority of them (14) have been previously treated with hydroxyurea (8 cases), for a mean treatment duration of 20 months (3-60 months), 6 with interferon for a mean duration of 26 months (2-60 months); two patients were previously untreated. STI therapy was started at the dose of 400 mg/day. During the first month of STI treatment hematologic response was obtained in 84% of cases, followed by major or complete cytogenetic response within the 6th month in 70%. Nine patients with good tolerance continued STI therapy at the initial dose, maintaining their hematologic and cytogenetic response. In 3 cases, in spite of STI dose reduction because of hematologic toxicity, WHO grade 3, hematologic and cytogenetic response was maintained. Four cases developed severe extra-hematological toxicity at CNS level (1 case) with STI discontinuation in 3 patients, while in 1 case STI was reduced to 200 mg/day with complete resolution of toxicity and continuous hematologic response. Out of the 3 patients who discontinued STI, 1 case developed blastic phase. In conclusion, STI treatment of elderly CML patients is justified, although the possible development of extra-hematological toxicity suggests the need of a close monitoring beside large clinical studies aimed to find the optimal dose for this subset of patients.

PO272
EFFECT OF THERAPY WITH IMATINIB ON PERIPHERAL BLOOD LYMPHOCYTES SUBSETS IN CHRONIC MYELOID LEUKEMIA PATIENTS
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Recently, Imatinib, a new selective inhibitor of bcr/abl derived tyrosine-kinase has produced exciting results in the treatment of chronic myeloid leukemia (CML) patients. Little is known on the effect of Imatinib on the immune system particularly after a prolonged treatment. In fact even if CML T cells do not have the t(9;22) hallmark translocation, about 25% of B cell lymphocytes and most of dendritic cells have been found Ph+ in CML patients at diagnosis. Thus, a selective inhibition of part of the antigen presenting cells compartment during treatment with Imatinib, could theoretically result in some sort of impairment of T cell arm. In the present study we evaluated the distribution of peripheral blood lymphocytes in 6 chronic phase CML patients at diagnosis, after a short treatment with Hydroxyurea and after 3, 6, 12 and 18 months of consecutive treatment with 400mg/day of Imatinib. At diagnosis, the percentage of CD3+, CD16+ and CD19+ lymphocytes in CML patients was similar to normal subjects (72.6±11.5% vs 78±10%; 11.6±4.3% vs 12±2; 4.5±3.9 vs 6±3, respectively) while the ratio CD4/CD8 was reduced both due to a sensible decrease of CD4+ (39.3±6.1% vs 54±5%) and an increase in CD8+ cells (34.3±6.1% vs 23±8%). When compared to normal individuals, in CML patients we found an increased percentage of CD56+ and CD57+ cells (18±7 vs 12±2 e 24±6, 8 vs 9±4, respectively) and with regard to CD4+ cells subset, we documented an evident altered distribution of CD45Ra and CD45RO (438±380/µL v.s. 649±362/µL e 696±78/µL). On the contrary, in CML patients the absolute number of CD45Ra was confirmed sensibly higher (804±291/µL v.s. 421±33/µL). The absolute number of CD16+, CD56+ and CD57+ lymphocytes (438±380/µL, 649±362/µL e 872±565/µL v.s. 264±44/µL, 220±66/µL e 198±88/µL, respectively) was found higher in CML patients while no difference were documented in B CD19+ cells total number. After a relatively short time of cytoreduction with Hydroxyurea (mean 20 days), while no substantial modification in lymphocytes percentage values was observed, a reduction of both overall lymphocytes population and relative subclasses was documented. Nevertheless, the decrease resulted statistically significant (p = 0.042) only for CD4/CD45RO, going from 804±291/µL down to 421±33/µL. The subsequent treatment with Imatinib did not appeared to negatively interfere with the percentage of various lymphocytes classes and subclasses. On the contrary, already after the first month of treatment, we could document a fair increase of percentage of CD4+ cells with a progressive recovery of CD45Ra and reduction of CD45RO, respectively, both reaching almost normal percentage values by the end of first year of therapy (53.3±9.6 vs 65±7 e 44±9 vs 42±11). Treatment with Imatinib didn’t vary neither the absolute number of T cells nor that of T cell subset, but despite a progressive increase of total peripheral blood lymphocytes, overall T cells classes and subclasses remain still significantly reduced even after one year after treatment, when compared to normal individuals. On the contrary, during treatment with Imatinib we documented a progressive increase of both percentage and total number of CD16+ and CD56+ lymphocytes that maintained a normal NK activity during all time points of the study. Although very preliminary, our results suggest that even up to 18 months of consecutive treatment, Imatinib didn’t induce any additional
The BCR-ABL gene of chronic myelogenous leukemia (CML) encodes for a cytoplasmic oncoprotein with constitutive tyrosine-kinase activity. Through its catalytic activity, BCR-ABL triggers multiple signal-transduction pathways that cause increased proliferation and decreased cell death. Imatinib mesylate (STI571) is a competitive inhibitor of the BCR-ABL tyrosine-kinase that abrogates BCR-ABL signaling, thereby leading to the death of CML cells. However, several reports have shown the occurrence of resistance to Imatinib both in immortalized cell lines and in CML patients. We describe a different approach for the use of Imatinib in the eradication of BCR-ABL-expressing cells. We previously reported that suppression of BCR-ABL kinase activity by Imatinib, could partially restore the nuclear translocation of the oncoprotein. By combining Imatinib with the nuclear export inhibitor Leptomycin B (LMB), we were able to coerce BCR-ABL inside the nucleus. Under these conditions, reactivation of BCR-ABL kinase activity induced cell death. Hence, nuclear entrapment of BCR-ABL may be able to selectively purge BCR-ABL-expressing cells from the bone marrow of CML patients. In order to evaluate the efficacy and selectivity of our strategy, we collected bone marrow and peripheral blood specimens from five healthy donors and thirteen CML patients. Mononuclear cells were isolated through a Ficoll gradient and then incubated in vitro for 24 hours with Imatinib, LMB or both drugs combined. Untreated cells were added as a control. At the end of treatment, the cells were plated on methylcellulose and scored after 3-4 weeks by evaluating the total number of BFU-E and CFU-GM. Randomly selected colonies were also screened for BCR-ABL expression by one-shot RT-PCR to establish if the combined use of LMB and Imatinib positively selected for BCR-ABL-negative cells. Specimens derived from healthy donors exhibited colony survival rates of 55% or 39% after Imatinib or LMB treatment, respectively. The two drugs combined produced a colony survival rate of 43%, suggesting that the combination of LMB and Imatinib caused limited levels of toxicity. Experiments performed on cells isolated from CML patients showed that the combination of LMB and Imatinib was extremely effective in reducing overall colony formation (p<0.0001). Moreover, treatment with the two compounds significantly reduced the number of BCR-ABL-positive colonies compared to both untreated cells (p<0.0001) or cells exposed to Imatinib alone (p=0.006). In summary, our two-drug strategy seems capable of stringently purging the bone marrow from BCR-ABL-expressing cells, thus raising the possibility that autologous bone marrow transplant might become a viable therapeutic option for several patients affected by BCR-ABL-positive leukemias.
soon as imatinib was re-introduced because of CML relapse. All patients were then treated with prednisone at daily doses of 25-50 mg for 4-8 weeks, this resulting in complete resolution of liver alterations. While on prednisone treatment all patients were re-treated with imatinib, starting from 100-200 mg/day up to full therapeutic dose of 300-400 mg/day, achieving again hematological +/- cytogenetic response. Prednisone was then tapered in 2-3 months in all patients and finally withdrawn in 2, while continuing imatinib therapy, without further hepatotoxicity. Our report suggests that imatinib-induced liver toxicity may be of immune origin, as suggested by delayed onset and response to prednisone. Corticosteroids allow imatinib therapy to be resumed at effective doses.

PO275  
IMATINIB RECRUITS HISTONE DEACETYLASE 1 FOR TRANSCRIPTIONAL REPRESSION OF GENES INVOLVED IN Deregulated PROLIFERATION AND RESISTANCE TO APOPTOSIS OF CHRONIC MYELOID LEUKEMIA HEMATOPOIETIC PROGENITORS  
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Changes in the chromatin structure, allowing the access for RNA polymerase to initiate transcription, are essential for the transcription of genetic programs. Accordingly, the activation of chromatin remodeling occurs prior to the induction of gene expression. The reversible acetylation of conserved lysine residues within the N-terminal tails of nucleosomal histones is the best studied chromatin modification. It is regulated by two antagonist types of enzymes, the histone acetyltransferases (HATs) and histone deacetylases (HDACs). In particular, the recruitment of class I HDAC (HDAC1, 2, 3 and 8) to DNA through specific DNA-binding complexes involving corepressors and silencers results in transcriptional repression of genes required for cell proliferation, differentiation and death. This is the story of bcr-abl-expressing cells (32D cell clones stably transduced a temperature-sensitive bcr-abl construct) that, in response to Imatinib, exhibited a significant increase of nuclear HDAC1 and a decrease of histone H4 acetylation. Changes in the enzyme nuclear expression and histone acetylation status were shortly followed by transcriptional downmodulation of unrelated genes, including p21 and Bax, and, more importantly, of bcr-abl itself. The process was conditional upon the drug-induced inhibition of p210 tyrosine kinase activity. In fact, it did not occur under culture conditions non permissive for the fusion protein constitutive activation. HDAC1 nuclear import and function are prevented by its improper cytoplasmic location resulting, in turn, from its binding to active p210 in a likely multimeric complex (including casein kinase, p53 and c-Myc). Both are restored by the Imatinib-induced inhibition of p210 tyrosine kinase. To conclude, the data presented here and results of previous studies allow us to assume a dual mechanism of action of imatinib on bcr-abl rearranged hematopoietic progenitors. The drug competition with the ATP-binding site of the abl catalytic subunit and deacetylase-catalyzed transcriptional repression of bcr-abl abrogate the survival signal, the p210 bcr-abl protein, which allows the illegitimate proliferation and extended survival of clonal myelopoesis. Both mechanisms appear to be critical for the drug's ability to control and possibly cure CML.

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PO276  
CDC25A OVEREXPRESSISON IN CHRONIC MYELOID LEUKEMIA PROGENITORS CAUSES RESISTANCE TO IMATINIB INDEPENDENTLY OF THE DRUG-INDUCED INHIBITION OF P210 BCR-ABL TYROSINE KINASE  
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In mammalian cells progression through the cell cycle is governed by cyclin-dependent kinases (Cdk) activated by their binding to cyclins. In particular, the transition from G0/G1 to S phase of the cell cycle, otherwise denominated the G1/S checkpoint and critical for regulated proliferation and protection of genomic integrity, is driven by the Cdk2-cyclin E complex, modulated, in turn, by small Cdk inhibitor proteins, including p21 and p27, and both positive and negative Cdk phosphorylation regulators, including the Cdc25 phosphatases. Cdc25 phosphatases remove inhibitory phosphates from specific tyrosine and threonine residues within the ATP-binding domain of Cdk proteins, thus activating them. Cdc25A, in particular, catalyzes the removal of Cdk2 inhibitory phosphorylation at Thr14 and Tyr15. Its ubiquitin-dependent/proteosome-mediated degradation by phosphorylation at Ser123, proceeding from activated Chk2, prevents the activating dephosphorylation of Cdk2 and causes a late G1/early S phase arrest. Our previous work supports the partic-
gene expression, and their roles in myeloproliferative disorders.
an abnormal size and morphology. The inability of dysplastic GATA-1low Mk to mature up to pit shedding would lead to a local increase of fibrogenic/osteoogenic cytokines eventually responsible for myelofibrosis development. The aims of this study were (i) to determine whether treatment with thrombopoietin (TPO) would restore GATA-1low Mk function increasing their platelet release, and (ii) to address the correlations between the defective Mk maturation in GATA-1low mice and the development of IM. GATA-1low mice 12-14 months old, and age-matched littermates (WT), were injected with pharmacological (25 μg/kg/d/10 days) or supra-pharmacological (100 μg/kg/d/5 days) doses of rmTPO (kindly provided by Kirin Brewery Co, Guma, Japan). Blood cell counts, number of Mks and of progenitor cells in the marrow, spleen and liver and extent of fiber deposition (by a semiquantitative method on Gomori-stained sections) were sequentially evaluated up to 21 days after the beginning of the treatment. TPO-treated WT animals showed a prompt (5-7 days) and sustained increase in ptl counts (>3-fold at the peak point with both schedules). Also in the case of GATA-1low mice, TPO-treatment increased ptl counts, but maximal responses were not observed until day 14-16 (high TPO dose) or 16-18 (low TPO dose) after treatment; however, maximal increases were even higher than those observed in WT animals (3 and 5-fold at low and high TPO dose, respectively). In addition, the majority of ptl circulating in GATA-1low mice after TPO-treatment presented a normal morphology and could be also found in discrete aggregates. The increase of ptl number was preceded by increases in the numbers of Mks (2-5-fold) and of CFU-Mk (800-1000-fold) in the liver and spleen of both WT and Mks (2-5-fold) and of CFU-Mk (800-1000-fold) in the marrow, spleen and liver of WT and GATA-1low mice; however, while a significant, although reversible (at day 21) increase in fiber deposition was observed in the marrow, spleen and liver of WT animals, GATA-1low mice showed no significant increase of fiber deposition in the bone and the spleen, and only a modest one in the liver (where the percentage increase of Mks had preferentially occurred after TPO-treatment). Interestingly, GATA-1 mRNA (by RT-PCR) was detectable in Mks purified from GATA-1low mice 24 hrs after discontinuing the TPO-treatment while GATA-1 protein (by immunohistochemistry) was detectable in Mks present in their tissues up to 21 days after treatment. Quantification of TGF-β1 mRNA and protein by RT-PCR and immunohistochemistry, respectively, in the spleen of TPO-treated mice showed and increase in the mRNA levels and the percentage of TGF-β1+ Mks in WT mice, while changes were of the opposite sign in the mutant mice. Therefore, TPO-treatment does not accelerate the progression of IM in GATA-1low mice and restores, at least partially, the plt number and morphology through a compensatory mechanism which involves induction of GATA-1 expression in the Mk; furthermore, these data strengthen the correlation between Mk hyperplasia/dysplasia and the development of IM in GATA-1low mice possibly mediated by TGF-β1.

PO279 BCR/ABL P190 POSITIVE CLONES ARISING IN PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS AND THEIR RESPONSE TO IMATINIB TREATMENT


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The rare occurrence of a BCR/ABL rearrangement as a secondary event in chronic myeloproliferative disorders (CMPD) with clinical features of atypical CML or CMML has been previously reported (Roumier et al., Haematologica 1999, 84:1075). We treated with imatinib two patients characterized by the late appearance of a p190 BCR/ABL rearrangement, in the setting of a preexisting Ph-negative myeloproliferative clone. Patient #1, a 62-year-old man, was diagnosed as having a idiopathic myelofibrosis in Oct 1997. At that time the presence of a Ph-chromosome was not detected, and the patient remained untreated and stable until March 1999, when white blood cells (WBC) started to increase up to 90.000/cm³ with a parallel drop in platelet count and increase in spleen size. Hydroxyurea treatment was started with partial hematologic remission. In Jan 2000 a molecular and cytogenetic analysis was repeated and the presence of a BCR/ABL transcript (p190 type) and a Ph-chromosome in 100% of the cells were observed. The patient was initially treated with α-IFN. After three months, hematologic remission was not obtained although the Ph+ clone, evaluated by FISH, decreased from 84% to 35% of the cells. He was shifted to receive imatinib treatment, which resulted in an initial improvement, soon followed however by a new worsening of the hematologic picture and progressive increase in the WBC and platelet counts. A BM done on day +90 was consistent with a myeloproliferative disorder without fibrosis. However, all mitosis had a normal diploid karyotype at standard cytogenetics and Ph+ cells at FISH were only 4% of the total. Hydroxyurea was added with the aim of controlling leukocytosis and thrombocytosis, but hematologic control remained unsatisfactory and imatinib treatment was stopped after 6 months. The patient continued on hydroxyurea for the following year, remaining cytogenetically in complete remission. In 2002 he died because of a myeloid blast crisis; again cytogenetic analysis was normal, 4.6% of the cells were Ph+ at FISH and the p190
transcript was still present at low levels. Patient #2 was diagnosed as essential thrombocytopenia in 1990; cytogenetic analysis showed a normal karyotype. A good control of the thrombocytosis was obtained with low dose hydroxyurea treatment, which continued for 10 years. From 2000, the patient refused hematologic follow-up and treatment, as the hematologic picture was stable. In 2003 he returned to the hospital for bone pain; the hematologic data showed leukocytosis (up to WBC 42,900/cm³), with immature myeloid cells and 16% monocytes in PB; platelets were in the normal range; spleen was enlarged. At bone marrow analysis increased myeloid series with fibrosis and monocytosis were observed; cytogenetics was normal but FISH disclosed the presence of a BCR/ABL rearrangement in 47% of interphase cells. RT-PCR was positive for a p190 transcript. The patient started therapy with imatinib, 400 mg/day, which induced a rapid decrease in WBC count; however, the platelet count rised; after 8 weeks of imatinib WBC were 4,760/cm³ and platelets were 977,000/cm³ and hydroxyurea was added again. Bone marrow was controlled at month 4 and showed the decrease of Ph⁺ cells to 7.4% of the cells. In both patient a p190 positive clone arised after a Ph-CMPD and partially replaced the Ph- clone (Ph⁺ cells at FISH 84 and 47% respectively). This clone was apparently well inhibited by imatinib treatment, which however allowed the reexpansion of the Ph⁻, BCR/ABL- clonal cells resulting in a overall poor hematologic control.

PO280
SERUM LEVELS OF ANGIOGENIN IN CHRONIC MYELOPROLIFERATIVE DISORDERS
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It has been established that angiogenesis contributes to disease progression in solid and hematopoietic malignancies. Consistently, increased microvessel density has been identified in the bone marrow of patients with hematologic malignancies, and elevated levels of circulating pro-angiogenic factors have been shown to correlate with stage and prognosis in a variety of hematologic disease. Angiogenin is a powerful one of these factors produced by neoplastic cells and/or host microenvironment. Recently, high levels of Angiogenin were found to correlate with a poor prognosis in patients affected by acute myeloid leukemia, myelodisplastic syndromes and lymphomas, but no data are available on soluble Angiogenin in Chronic Myeloproliferative Disorders (CMD). Aim of this study was to investigate levels of sAngiogenin (Ang) and soluble Transforming growth factor β (sTGF-β1) in patients with CMD, correlating them with clinical variables and activity disease. sTGF-β1 was chosen because of its repeatedly demonstrated role in organ fibrosis and carcinogenesis. Enzyme-linked immunosorbent assay detected (p<0.05) higher levels of sAngiogenin in CMD compared to healthy subjects (1031.6±374 pg/mL and 195.7±39.8 pg/mL, respectively). The highest levels of sAngiogenin were detected in CML patients (1349.36±549.54 pg/mL); but they correlated neither with blast count nor with white blood cells count. Noteworthy, CML patients who achieved hematologic remission after Interferon therapy showed circulating levels of Angiogenin significantly (p<0.05) decreased compared those at diagnosis. In ET patients levels of Angiogenin (889.36±267.66 pg/mL) and sTGF-β1 (76.69±6.08 pg/mL) were higher (p<0.05) compared to healthy controls (57.93±3.99 pg/mL). Interestingly, significantly (p<0.05) higher levels of sTGF-β1; were detected in ET patients with history of thrombotic episodes, compared to those of patients without history of thrombotic complications. No correlation was found between levels of sAng and levels of sTGF-β1 or platelet count among ET patients. Our results show for the first time that elevated blood levels of Ang feature chronic myeloid malignancies suggesting a role of Ang in the pathogenesis of these diseases.

PO281
SUCCESSFUL TREATMENT OF ADVANCED IDIOPATIC MYELOFIBROSIS WITH IMATINIB MESYLATE SODDISFACENT TRATTAMENTO DI MIELOFIBROSI IDIOPATICA IN TRASFORMAZIONE CON IMATINIB MESYLATE
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Idiopathic myelofibrosis (IM) is a clonal myeloproliferative disorder characterized by bone marrow fibrosis, extramedullary hemopoiesis, splenomegaly and leukoerythroblastic blood picture. Up to date, conventional treatment for IM has been directed toward the alleviation of symptoms and has shown limited efficacy without improving overall survival. As such, investigations on new therapeutic strategies are warranted. We here describe two cases of IM in transformed stage of disease who were successfully managed with imatinib mesylate. The patients were two males, age 63 and 59 years respectively, whose diagnosis of IM was confirmed by standard criteria. Case 2 presented hepatic cirrhosis also. Cytogenetic analysis revealed in both cases a normal karyotype and there was no finding of molecular Bcr-Abl. The two patients underwent conventional treatment with hydroxyurea but become drug-refractory after a mean time of 21,5 months. Both cases showed uncontrollable hyperleukocytosis (mean
WBC-count: 59.8×10^9/L and PB morphologic features of accelerated phase), case 1 had a decreased Hb-concentration (Hb: 8.9 grams/deciliter), while case 2 needed weekly red blood and platelet cell transfusions because of anemia (Hb: 7.2 grams/deciliter) and severe platelet reduction (PLT: 9.0 ×10^10/L). The pictures of bone marrow aspirates confirmed diagnosis of accelerated phase of the disease. Cytogenetic re-evaluation showed no clonal abnormalities in case 1 while case 2 presented monosomy 7.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Before imatinib mesylate therapy</th>
<th>After imatinib mesylate therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count (×10^9/L)</td>
<td>58.5</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Case #1

- Hb concentration (g/dL): 8.9 → 11.9
- PLT count (×10^9/L): 544.0 → 349.0
- WBC count (×10^9/L): 61.2 → 16.2

Case #2

- Hb concentration (g/dL): 7.2 → 11.2
- PLT count (×10^9/L): 9.0 → 104.0

Therapy with imatinib mesylate, at a daily dosage of 400 mg, was then started. Both patients exhibited a prompt hematologic response. Imatinib mesylate normalized or reduced the WBC-count (case 1, WBC: 10.8 ×10^9/L; case 2, WBC: 16.2 ×10^9/L, respectively), led to both an increase in Hb-concentration and platelet count (normalizing them), and reduced the degree of hepato-splenomegaly. Imatinib mesylate administration is still ongoing with minimal side effects. The therapeutic role of imatinib mesylate therapy in chronic myeloproliferative disorders other than CML remains to be explored. Since it has been reported that IM frequently harbour either activating mutations of the kit-gene or rearrangement of the PDGFRs, our clinical results might lead to hypothesize an attractive and promising role for imatinib mesylate in the treatment of a subset of IM.

EL is a proliferative disorder diagnosed when hypereosinophilia is associated with clonal cytogenetic abnormalities. Indolent or aggressive clinical course are described, and there are not pharmacological resources capable of curing the disease. We have treated two young patients affected by EL by imatinib, the bcr/abl tyrosine kinase inhibitor, with very good results. The first patient is now a 32 year old man, whose disease began in 1991, when CEL was diagnosed in our unit. Splenomegaly and hypereosinophilia (8000/mm3) with dysplasia (ring hole nuclei, abnormal basophilic granules) were his clinical and hematologic features at diagnosis. The karyotype was 46XY t(1;5)(q23;q31). The patient was treated by alfa-interferon for many years, attaining complete hematologic remission and a major cytogenetic responses. In April 2001 the treatment was interrupted for intolerance (headache and vomiting). In May 2002 he started imatinib 400 mg/d, kindly provided for compassionate use by Novartis Pharma: after 15 days, eosinophils drastically reduced (eos: 180/mmc), in absence of any side effect. The dosage was then reduced because of leukopenia, and the patient is now taking 200 mg/d and has normal hemogram and spleen size. A recent cytogenetic analyses showed the following karyotype: 46XY t(1;5)(q23;q31) [2] / 46 XY [8]. The second patient, a 36 year-old man, came to our observation in December 2002 because of hypereosinophilia and cardiomiopathy. Thrombocytopenia (plt. 54.000/mm^3), hypereosinophilia (WBC 23.000/mm^3 eos. 14.000/mm^3) with some ring-hole eosinophils, and increased LDH (526 UI/mL) were detected by basal work up. The echocardiography revealed severe infiltration of left ventricle wall, involving also tendons and muscles of the mitral valve. Bone marrow cytology confirmed eosinophil hyperplasia with dysplasia, and cytogenetic analysis performed on 24 h unstimulated culture showed 46 XY add 17 q(25) in all 20 metaphases observed. Interphase fluorescence in situ hybridization, performed with BAC 3H20 for the 3’ FIP1L1 and the CH1C2 genes, showed monosomy in 44% of nuclei, typical of chromosome 4 q12 region deletion. RT-PCR and nested PCR amplification, performed on peripheral blood sample using FIP1L1 and PDGFRα specific primers, showed the presence of the FIP1L1/PDGFRα fusion gene both in first round and in nested amplifi-
cation. Anti-coagulation by dicumarol and imatinib 200 mg/d were started. One week after, eosinophil count fell to 150/mm³ and platelet rose to 132,000/mm³. After one month, imatinib was reduced to 100 mg/d without any adverse effect. He has now a normal hemogram (Hb 12.4; WBC 5500/mm³; eos. 90/mm³; plt. 215000/mm³), and there are echocardiographic signs of marked improvement of endomyocardial infiltration. Bone marrow eosinophils are about 10% and cytogenetic and molecular analyses show a major cytogenetic response (only 2 out of 12 observed metaphases show add 17q), FISH analysis shows 98.7% of nuclei with two hybridization signal, only the nested PCR detects the fusion gene. Our and others’ experience with imatinib in eosinophilic proliferations is extremely promising: rapid eosinophil normalization employing low doses of the inhibitor; possibility of cytogenetic remission, disappearance of cardiac infiltration and absence of significant side effects are therapeutic potentialities not shared by other pharmacological agents. These anecdotal experiences should be the basis for further controlled studies.

PO283
RESPONSE TO IMATINIB IN CHRONIC MYELOPROLIFERATIVE DISEASES WITH ACTIVATION OF THE PLATELET DERIVED GROWTH FACTOR β RECEPTOR (PDGFR): A NEW CASE REPORT
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Based on its ability to inhibit the tyrosine kinase activity of ABL, as well as the c-kit and the Platelet Derived Growth Factor Receptor tyrosine kinases, the spectrum of diseases that may respond to Imatinib is increasing. A recently recognized subgroup of myeloproliferative disorders/myelodysplastic syndromes (MPD/MDS) has a t(5;12)(q33;p13) with the activation of the gene for PDGFR which encodes a receptor tyrosine kinase. Here, we present the case of a patient, with MPD/MDS, and eosinophilia, carrying a translocation t(5;12)(q33;p13) who achieved a complete remission following treatment with Imatinib, 400 mg daily. At the time of writing he still remains in complete remission with an excellent performance status. Very similar clinical pictures were seen in other patients who had diseases characterized by primary deregulation of PDGFR and to date four additional partner genes (H4, HIP1, CEV14 and Rab5) have been reported. At the molecular level almost all of these translocations result in a constitutive activation of protein tyrosine kinases. So far, to our knowledge, four cases with t(5;12)(q33;p13) and one patient with CMML with t(5;17)(q33;p13)have been treated with STI571 and reported in the literature. All had prompt responses with normalization of the blood count, disappearance of eosinophilia, resolution of cytogenetic abnormalities. There is clearly a need for further studies of Imatinib in MPD/MDS with chromosomal translocations involving PDGFR to confirm these promising initial results.
Hepatic iron concentration and total body iron stores in genetic hemochromatosis and thalassemia major

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To characterize quantitatively differences in the sites of storage iron deposition in genetic hemochromatosis and thalassemia major, we compared studies of 87 patients homozygous for the C282Y mutation in HFE with those of the 54 patients who had undergone successful allogeneic bone marrow transplantation for thalassemia previously described (N Engl J Med 2000; 343:327-31). Before beginning phlebotomy therapy, iron was measured in specimens of liver obtained by percutaneous biopsy and records were then kept of the amount of blood removed until body iron stores were depleted. To maintain comparability with the results of Angelucci et al., we excluded patients with cirrhosis (n = 12) or with liver samples obtained by biopsy weighing less than 1.0 mg, dry weight (n = 55) and calculated the magnitude of total body iron stores similarly. In brief, total body iron stores were calculated from the total amount of blood removed, assuming that each gram of hemoglobin contains 3.4 mg of iron, with adjustment for any change in the concentration of circulating hemoglobin, assuming a blood volume of 61.9 mL/kg for women and of 62.4 mL/kg for men. In women with regular menses, an iron loss of 0.5 mg per day was assumed during each month of menstruation. Because of uncertainty about the quantitative extent of variation in dietary iron absorption both within and between patients, no adjustment was made for an increase in iron absorption during phlebotomy but sensitivity analyses were carried out to estimate potential effects.

The Figure shows the results of linear regression analysis between the initial hepatic iron concentration and calculated total body iron stores (i) in our 20 patients with genetic hemochromatosis and liver samples that were at least 1.0 mg in dry weight (filled circles) and (2) in the corresponding 25 patients with thalassemia major after successful transplantation (open circles).

For the patients with genetic hemochromatosis, the estimated slope of the regression line was significantly different from zero (t=5.3, 18 df, p<0.0001) but the estimated intercept was not significantly different from zero (t=-0.02, 18 df, p=9.98). The estimated slope of the regression line for the patients with genetic hemochromatosis was significantly less than that for patients with thalassemia major (F=26.1, 1 and 41 df, p<0.0001). In a further regression analysis with the assumption that hepatic iron stores were reduced to zero with phlebotomy therapy, variation in the hepatic iron concentration in the patients with genetic hemochromatosis accounted for more than 90% of the variation in body iron stores and was not appreciably affected by proposed models of the increase in iron absorption during phlebotomy. Overall, this relationship could be expressed as

Total body iron stores = 5.2 × Hepatic iron concentration [Genetic hemochromatosis]

while the corresponding relation found by Angelucci and colleagues was

Total body iron stores = 10.6 × Hepatic iron concentration [Thalassemia major]

with body iron stores expressed as mg/kg body weight and hepatic iron as mg/g liver, dry weight. These results provide a quantitative estimate of the magnitude of the difference in the distribution of excess iron between the liver and extrahepatic sites in the two conditions. For a given hepatic iron concentration, the body iron excess in thalassemia major is about twice that in genetic hemochromatosis.
PO285
THE ITALIAN REGISTRY OF FANCONI ANAEMIA (RIAF): 2003 REPORT
D' Amico F,^ Montone E, ^ Calzone R, ^ Zatterale A ^ on behalf of Riaf Contributors


Fanconi's anemia (FA) is a rare genetic disease, characterized by progressive pancytopenia, malformations, cancer proneness and chromosomal instability. At least seven different groups (FA-A to FA-G) have been defined by complementation tests. The FA patients can be misdiagnosed because of the clinical heterogeneity, the variable natural history, and the skills required to carry out the specific diagnostic test, showing chromosomal instability (DEB test). Because of the clinical variability and genetic heterogeneity, the scientific research is even more difficult to design in FA than in other rare diseases. The Italian Registry of Fanconi Anaemia (RIAF), collecting both clinical and epidemiological data, is an invaluable tool to improve the knowledge about the clinical features, the natural history, the diagnosis and treatment of this syndrome. It was designed since 1994 at the Department of Genetics of 'ASL Napoli 1' in Naples, where a number of Italian FA patients obtained genetic counseling and diagnostic tests in the last decades. FA patients are referred to RIAF by many physicians whom the AA. are very grateful to. FA patients have been enrolled in the Registry with the condition that they were positive for chromosomal instability, both spontaneous or DEB induced, according to Auerbach's protocol. Most of the enrolled patients had the DEB test performed at the Cytogenetics Unit of the Genetics Department in Naples. The patients signed a consent form as to the treatment of their personal data and to give blood samples for genetic tests. Their personal data are invariably treated anonymously. At 2003 the RIAF has records of 123 patients, 70 alive with ages varying from 0 to 39y. The patients' ages at the onset of hematologic symptoms range from 1m to 26y, whilst at diagnosis the age ranges from 1m to 33y. Malformations are reported in 104 patients, malignancies in 9 (5 having had a stem cell transplantation before the malignancy onset). 47 patients underwent bone marrow transplantation, 26 of them from consanguineous donors (of which 20 are alive and 6 dead), 21 from unrelated donors (of which 4 are alive and 17 dead). The study of patients' origin was very interesting. Two geographic clusters were located in the North-East of Italy and in the Campania region (Benevento area). The possibility of a referral bias could be considered, however a body of evidence suggests a real prevalence excess in the above mentioned areas. 15 Italian FA families underwent cell fusion studies for the identification of the complementation group (Joenje H, Amsterdam). 11 of them were classified as belonging to complementation group A and 1 to group G, 3 showing 'resistance' to MMC test. Studies are in progress to identify the complementation group in Italian FA patients by faster methods. In the last years the RIAF has been giving a precious support to FA research, participating to a number of European and USA research projects on FA epidemiology, pathophysiology and to studies on phenotype-genotype correlation (Faivre et al, 2000; Demuth et al, 2000).


PO286
CARBOXY-TERMINAL FRAGMENT OF OSTEOGENIC GROWTH PEPTIDE ACTIVITY ON ESSENTIAL THROMBOCYTHEMIA
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Essential thrombocythemia (ET) is a myeloproliferative disorder characterized by an increased level of platelets in peripheral blood and an excessive number of megakaryocytes in bone marrow. The increase of megakaryocytes and platelets appears to be secondary to a deregulation of homeostatic mechanisms of megakaryocytopoiesis including increased sensitivity to stimulatory cytokines, reduced response to inhibitory stimuli or independence from growth factors. Osteogenic growth peptide (OGP) has been shown to increase blood and bone marrow cellularity and to enhance engraftment of bone marrow transplants in

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PO287

STABLE RETROVIRAL TRANSDUCTION AND EXPRESSION OF HUMAN CD20 GENE IN NK-92 CELL LINE: A POSSIBLE INNOVATIVE TOOL FOR CELLULAR IMMUNOTHERAPY

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NK-92 is a highly cytotoxic, IL-2 dependent cell line derived from a non Hodgkin Lymphoma patient. This cell line shows the typical immunophenotype of an activated CD3 negative, CD56 positive NK cell and exhibits substantial antitumor activity against a wide range of malignancies in vitro as well as in xenograft SCID mice. On the basis of these properties and due to the lack of any measurable in vivo tumorigenic activity, NK-92 cells have been proposed for ex vivo purging of hematopoietic stem cells prior to autografting in CM L and AML patients and more recently also entered a Phase I-II clinical trial for the treatment of patients with advanced solid tumors (Tonn et al.: J Hematother 2001;10:535). Although the in vivo use of these unrelated cytotoxic leukemic cells has not been so far associated with detrimental side effects for the host, many aspects about the safety of such an approach still remain to be addressed. One possible effective strategy to control an undue in vivo outgrowth of these effector cells or their cytotoxic activity against non neoplastic cells is to genetically modify NK-92 cells with a suicide gene. Under this respect, we have recently developed an innovative gene suicide strategy based on the possibility of transducing the complete cDNA of the human CD20 into normal T lymphocytes. This gene at the same time allows to drive the immunoselection of the infected cells and rendered these cells susceptible to the antibody-mediated lysis using the chimeric anti CD20 Monoclonal antibody Rituximab. Using a human cPPT/CTS lentiviral vector, the human CD20 gene was efficiently transduced into NK-92 cells. After repeated immunoselection procedures a stable CD20 positive NK-92 cell line was obtained. After more than 10 months of in vitro culture, these genetically modified cells did not show any change in their immunophenotypic characteristics, cytotoxic potential against several neoplastic cell lines and retain the same absolute IL-2 dependency for growth. Both parental and CD20 transduced NK-92 cells did not show a significant cytotoxic activity against human purified normal CD34+ cells, however preliminary data indicate that both cell lines inhibit day 14 normal CFU-GM but did not prevent the growth of day 21 CFU-GEMM colonies. Upon in vitro exposure to Rituximab (350 mg/mL) and 10% rabbit complement genetically modified CD20+ NK-92 cells were rapidly and completely killed, while no cytotoxic effect was registered when the parental cell line was used. Experiments are currently ongoing in a SCID mice model.
model we previously described and in which NK sensitive acute leukemia can be efficiently grown.

PO288
GENERATION OF IDIOTYPE-SPECIFIC T LYMPHOCYTES FROM HEALTHY DONORS AND ENRICHMENT THROUGH AN INTERFERON-γ CAPTURE ASSAY: IMPLICATIONS FOR ALLOGENIC ADOPTIVE IMMUNOTHERAPY IN MULTIPLE MYELOMA PATIENTS
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High-dose chemotherapy followed by autologous stem cell support represents an advantage over conventional chemotherapy in the treatment of multiple myeloma (MM), but only allogeneic bone marrow transplantation may eradicate the disease. In MM patients, adoptive immunotherapy through allogeneic donor lymphocyte infusions has been largely unsuccessful. The idiotype (Id) expressed by MM cells can be regarded as a tumor-specific antigen and it has been used for immunotherapy. In this study, we investigated the possibility to enhance the Id-specific antitumor effect of allogeneic T cells by using an ex vivo T-cell culture system followed by the purification of IFN-γ-producing T cells. For induction of T cells, peripheral blood mononuclear cells (PBMNC) from healthy donors were coincubated with autologous monocyte-derived dendritic cells (DC) generated in the presence of GM-CSF and IL-4 and pulsed with patient-derived Id protein. Cells were maintained in serum-free medium and supplemented during the priming phase with IL-7 and IL-12. Subsequently, the T-cell culture was restimulated every 7 days with pulsed DC in the presence of low doses of IL-2. After each restimulation, T cells were analyzed for intracellular IFN-γ by flow cytometry. After 2 stimulations, the percentage of Id-specific T cells was as high as 5%, whereas IFN-γ production by not stimulated T cells was undetectable. Based on their IFN-γ production, T cells were isolated by using a commercial immunomagnetic IFN-γ capture assay. The purity of enriched IFN-γ-producing T cells ranged between 30% and 50% as evaluated by flow cytometry. The yield was 60% of IFN-γ-positive T cells before selection and cell viability after selection was 100%. These data demonstrate that Id-specific T cells may be generated from healthy donors and significantly enriched on the basis of their IFN-γ production.

PO289
HUMAN BONE MARROW-DERIVED MESenchymAL STEM CELLS DIFFERENTIATE INTO NEUROGLIAL CELLS AFTER TRANSPLANTATION INTO NEWBORN MOUSE BRAIN
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Whole bone marrow and mesenchymal stem cells (MSCs) have been shown to differentiate into neuronal cells when infused into immunodeficient mice (Mezey 1997) or transplanted in rat brains (Azizi 1998, Kopen 1999). More recently, Mezey et al. (2002), analyzing postmortem brain samples from patients who had received bone marrow transplant, demonstrated that adult human bone marrow cells can enter the brain and generate neurons. We now report our preliminary results aimed to determine whether human MSCs can adopt neural cell fates when transplanted in the newborn mice. Human mesenchymal stem cells were obtained from healthy donors for allogeneic bone marrow transplants and expanded in culture for five passages by plastic adherence technique. Cells were transplanted into the brain cortex of newborn C57BL mice (4 and 7 days old) and characterized 7 and 30 days after transplantation. Tissues were analyzed by immunocytochemistry, RT-PCR and FISH for the presence of human and/or differentiated cells. Brains analyzed seven days after the transplant were negative for the presence of human neural markers but positive for high affinity NGF receptor. However, thirty days after transplantation, we showed that human MSCs migrate into the periventricular zones of host brain up to ~1-1.5 mm from the graft cortical core, and showed differentiation into both neuronal and glial phenotypes. Most of injected MSCs expressed neuroglial phenotypes and markers such as neurofilament 160Kd and GFAP. FISH analysis, performed on frozen and fixed sections using a probe consisting of a selection of alphoid sequences that hybridizes to the centromeres of all human chromosomes, confirmed the human origin of the differentiated cells. Moreover, RT-PCR on the same tissues was positive for both human GFAP and GAP43. Further experiments are needed to evaluate the frequency of this phenomenon and its functional relevance.
PO290 SAFETY OF RETROVIRAL GENE MARKING WITH A TRUNCATED NGF RECEPTOR


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Random integration into the host cell genome and inappropriate transgene expression are major safety concerns for the clinical use of retroviral vectors. Li and colleagues (Li et al. Science, 296: 497, 2002) have recently reported a leukemic transformation of murine bone marrow (BM) cells caused by integration of a retroviral vector into the Evi1 proto-oncogene, and suggested that expression of the transgene carried by the vector, a truncated version of the p75 low-affinity nerve growth factor receptor (LNGFR) contributed to the leukemogenic progression. Since LNGFR is used as a surface marker in gene therapy clinical trials aimed at controlling graft-versus-host disease (GVHD) after bone marrow transplantation, a critical assessment of the potential risks associated with the use of such molecule is essential. We, as seventeen independent groups of scientists, have accumulated both pre-clinical and clinical evidence supporting the safety of LNGFR as a cell marking molecule. Cumulative data obtained from >300 mice transplanted with BM cells transduced with retroviral vectors expressing LNGFR showed normal engraftment, persistence and differentiation of LNGFR+ hematopoietic stem/progenitor cells (HSCs) in primary, secondary and tertiary BM T recipients, and no adverse events. Over 100 of these mice were monitored for >20 wks, and more than 50 animals, including 16 recipients subjected to secondary or tertiary BM transplantation, were monitored for >28 weeks after BMT. Considering that a total of >1×10³ transduced cells were transplanted, and assuming an average of one retroviral integration/cell, we estimate the risk of oncogenic transformation following transduction with a retroviral vector carrying a LNGFR gene to be <1 in 10⁶ integration events. Therefore, expression of LNGFR did not increase the expected frequency of an insertional onco-genesis event, previously estimated at 10⁻⁸ to 10⁻⁹ per insertion event. In pre-clinical models of post-HSCT GVHD, no difference in the ability to induce donor chimerism or to mediate GVHD was observed for LNGFR+ vs control T-cells in 356 mice, 200 rats, and 3 dogs, again in the absence of any adverse event. Analysis of 102 independent transductions of human peripheral lymphocytes LNGFR-carrying vectors revealed no change in the expression of markers of lineage, activation or adhesion, nor in the proliferative capacity of T cells. All cells remained strictly dependent on IL-2 for growth and survival, and addition of potentially stimulatory doses of 50-100 ng/mL of NGF did not induce cell proliferation, expression of the CD25 activation marker, or secretion of TNF. Most importantly, no toxicity or other adverse effect has been associated with the use of LNGFR as a surface marker for genetically modified lymphocytes in phase-I clinical studies aimed at treating or preventing post-BMT GVHD. In 31 patients treated with donor lymphocytes transduced with two different vectors (SFCM M-2 or SFCM M-3), engraftment (up to 40% of circulating mononuclear cells) and long-term persistence (>82 months) of transduced cells was observed. Not a single acute or chronic adverse or toxic event related to the gene transfer procedure or to the transgene expression was observed during these trials, which involved infusion of >10₁¹ cells generated by >50 independent transductions. Taken together, these pre-clinical and clinical studies provide evidence that the use of LNGFR as a cell surface marker is safe, non toxic and non-tumorogenic in both mice and men.
Clinical prognostic models for adult ALL suffer from lack of precision, since about 40-50% of standard-risk (SR) patients may relapse whereas 20-40% of high-risk (HR) ones do not, which implies a definite risk of exposure to under- or over-treatment, respectively, including stem cell transplantation. NILG study 09/00 was undertaken to investigate the role of molecular MRD as a better prognostic indicator and primary decisional factor for the definition of postremission treatment intensity, which could vary from standard post-consolidation maintenance (MRD negative) to allo-genetic stem cell transplantation (MRD positive, with sibling donor) to post-consolidation intensification with four hypercycles followed by further low-dose maintenance. This report evaluates the interim results from the centralized laboratory evaluation of MRD. The molecular study consisted of three distinct phases: (i) the cloning of patient-specific probe(s) at diagnosis; (ii) the subsequent determination of MRD risk class at end of early consolidation, to assist in the choice of MRD-oriented treatment; (iii) the long-term monitoring of MRD. For step (ii), three sequential bone marrow samples were obtained before chemotherapy cycles 4, 6 and 8; these three samples were then analyzed together for MRD by means of PCR analysis of chimeric genes or patient-specific IgH/TCR-γ/δ rearrangements (with dot-blot), and by RQ-PCR (Taq-Man) since April '02. MRD negativity was defined, using any probe with a sensitivity >10^-3, by a BM sample 2 negative/very low (12 clinical standard risk = 6 MRD negative and 6 positive; 33 clinical high risk = 16 MRD negative and 17 positive). The relapse rate was 18% in MRD negative group and 61% in MRD positive group. Step (3): The MRD follow-up study is ongoing. Study feasibility was confirmed, with a high initial success rate and a relatively high applicability rate at time of MRD-based treatment decision. MRD is a useful and reliable indicator of the risk of relapse in individual patients with ALL and is therefore suitable to assist the clinician in the design and the interpretation of therapeutic trials.
INCIDENCE OF MLL GENE REARRANGEMENT IN ACUTE LEUKEMIA WITH 11q23 ABERRATION

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Structural abnormalities of the 11q23 band with MLL gene translocation is a recurrent chromosome change in leukemia and a recent proposal by the WHO specifies a separate category for AL with 11q23+/-MLL-. Conversely the outcome of patients with 11q23+ is not well defined; this possibly reflects the marked heterogeneity of 11q23 aberrations. As combining cytogenetic and molecular methods may reveal discrepancy between 11q23+ and MLL+ cases (Ibrahim et al. 2000) the definition of diagnostic methods may have clinical implications. Three-hundred-thirty newly diagnosed AL patients were analysed with CC and FISH using a commercial probe for the 11q23 locus (Vysis Inc.) that should detect all MLL translocation. Leukemia was diagnosed between January 1992 and November 2002 and classified according to the FAB Criteria: 290=AML, 44= ALL and 3=biphenotypic AL, median age=48 years (range 5-81years). Samples were processed following standard cytogenetic procedures after direct and synchronised short term cultures. Karyotypes were classified according to the ISCN 1995 Nomenclature. FISH was carried out on cytogenetic pellets (stored at -20°C in Carnoy’s solution) following established protocols (von Bergh et al. 2000). In 15 patients, who were 11q23-/MLL-, also M-ulti-Colour-FISH karyotyping (M-FISH) was done (Spectra-Vysion, Vysis Inc.)CC showed clonal abnormalities in 44.5% (n=147/330), normal karyotype in 40% (n=130/330) and failed in 16% (n=53/330). 11q23 rearrangement was observed in 7.2% of cases with assessable CC (n=20/277). FISH detected MLL rearrangement in 5% (n=17/330); 15 translocations (2 missed by CC because of failure) 1 amplification and 1 deletion. M-FISH identified a cryptic t(9;11)(p21;q23-24). Overall FISH, CC and M-FISH detected 11q23+/MLL+ rearrangements in 23 patients (6.9%); 20 AML, 2 ALL, and 1 biphenotypic AL. Truly cryptic MLL/11q23- cases were not found, but the combining of CC and FISH allowed to define better MLL involvement in 6 patients (26%), while in other 6 patients of this group, FISH ruled out MLL gene rearrangement (i.e. t(2;11)(p21;q23); t(11;16)(q23;p13); t(11;15) (q23;q12); t(11;12)(q23-24;q24); add(11)(q23); inv(9)(9;11)(p21;q23-24)). Following literature search we found a few comparable studies which report an incidence of 11q23-/MLL- (detected by FISH) between 7% and 22% and an incidence of 11q23+/MLL- cases within 0% and 40%. These discrepancies point out that the sensitivity of CC for identifying 11q23- is highly variable. This further supports the notion that molecular methods should be routinely used in combination with CC to identify better MLL+ cases and to distinguish typical 11q23-/MLL+ from 11q23 rearrangement without MLL translocation (Tanaka et al. 2001).
Fanconi anemia (FA) is an autosomal recessive disease characterized by congenital malformations, progressive bone marrow failure, high predisposition to cancers, and chromosomal instability. Seven FA genes have so far been identified: FANCC, FANCA, FANCQ, FANCE, FANCF, FANCD2, and BRCA2. Although their function is not clear, the FA proteins participate in a newly identified common pathway. Briefly, FANCA, FANCQ, FANCE, and FANCF interact with each other in a multimeric complex prior to the FANCD2 associated activity. In normal cells, the native FANCD2-S (small) isoform is monoubiquitinated to the FANCD2-L (large) isoform after DNA damage and targeted to nuclear foci. In FA subtypes A, B, C, E, F, or G, the FANCD2 monoubiquitination does not occur and FANCD2 does not migrate to foci. On these bases, we have developed FA protein test to classify patients to the different groups avoiding the complementation analysis that was fundamental for molecular diagnosis. We prepared several antisera against the FA proteins and demonstrated their specificity. The monoubiquitinated form of FANC-D2, which reveals a correct activity of FA complex, was detected together with the native FANCD2-S in all wild type cells whereas FANCD2-S was the only isoform present in lymphoblastoid cell lines with defects in the other FA proteins. We also found two FA cell lines with altered levels (absence or strong reduction of both isoforms) of FANCD2, suggesting that FA in these patients were due to mutations in the rare complementation group FANCD2. Sequencing analysis allowed us to ascertain the FANCD2 mutations, as well as to confirm the feasibility of the protein test to define which gene is defective in FA patients. A combination of immunoblotting against FANCD2 and the most frequent defective proteins FANCA, FANCQ and FANCQ should provide a powerful means to subtype and diagnose the majority of FA patients. This approach would also represent a feasible screening procedure for the patients in which a diagnostic suspicion of FA is legitimate on the basis of specific clinical signs, including aplastic anemia, cancers, and particular sensitivity to radiation and chemotherapeutic treatments.

8-hydroxy-2-deoxyguanosine (8-OHdG) in WBC and in urine. A highly significant deficiency in Trx plasma levels was observed in untransplanted FA patients (p<0.0005), consistent with the in vivo finding by Kontou et al. (2002). Trx deficiency was less pronounced in post-BMT patients. A significant decrease in TG levels, along with an increase in the GSSG/GSH ratio was observed in untransplanted patients vs. either FA heterozygotes or controls (p< 0.05). M Glx levels were significantly increased in untransplanted FA patients and even higher in FA heterozygotes compared to post-BMT FA patients, and vs. controls (p< 0.0037). A significant excess of 8-OHdG levels was observed in WBC from untransplanted FA patients (p< 0.04), to a greater extent in female vs. male patients (confirming the report by Degan et al. 1995); however, no significant excess of 8-OHdG in WBC was detected in post-BMT FA patients. Urinary 8-OHdG levels were found unchanged (with a non-significant increase) in FA patients compared to controls. Unlike the above parameters, 8-epi-PGF plasma levels showed a significant increase (p<0.005) in post-BMT, not in untransplanted FA patients. No significant changes were detected in plasma levels of Vitamin C, Vitamin E, or urate. An in vivo prooxidant state in untransplanted FA patients was assessed by multi-parameter analytical evidence, including a dramatic deficiency of Trx, and significant excess levels of MGlx, glutathione imbalance, and excess WBC (not urinary) levels of 8-OHdG. On the other hand, 8-epi-PGF plasma levels were increased in post-BMT FA patients. The excess MGlx levels observed in FA parents, higher than in patients, deserves further investigation as a potential diagnostic tool for the FA heterozygous state. The multi-faceted alterations in oxidative stress parameters provide compelling evidence for the in vivo involvement of redox abnormalities in FA clinical phenotype, expected to open novel research lines and a reappraisal of the clinical management of FA patients.

PQ295 DETECTION OF MUTATIONS OF THE RARE COMPLEMENTATION GROUP FANCD2 BY A NEW DIAGNOSTIC APPROACH OF FANCONI ANEMIA
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PQ296 CLONAL IMMUNE RESPONSES IN BONE MARROW FAILURE: EVIDENCE FOR A SEMI-PUBLIC AUTOIMMUNE PATHOPHYSIOLOGY AND ROLE IN CLINICAL PRACTICE
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An immune-mediated destruction of bone marrow progenitors is involved in most aplastic anemia (AA) and other related bone marrow failure syndromes. We...
systematically studied the immune repertoire in patients with bone marrow failure, looking for clonal T cell responses reflecting a pathogenic antigen-driven immune response. A total of 54 patient was analyzed, 45 with AA (14 with a significant paroxysmal nocturnal hemoglobinuria [PNH] clone: AA/PNH syndrome), and 9 with pure hemolytic PNH. The immune repertoire was analyzed at the level of the T cell receptor (TCR)-β chain, by flow cytometry and molecular techniques, including CDR3 size analysis (spectratyping), cloning and sequencing of the CDR3 motifs. By flow cytometry, we documented that aplastic patients present in both CD4 and CD8 compartments an abnormal utilization of TCR-β-variable regions (Vβ), with some Vβ subfamilies over-expressed compared to the normal range. CDR3 pools from the expanded Vβ subsets were amplified by RT-PCR using a common constant (Cβ) and the specific Vβ primers; Vβ families showing a skewed CDR3-length patterns were cloned in bacteria and single colonies were sequenced. Only CD8+ expansions were selected as suspicious for clonality. Expanded Vβ CDR3 pools from aplastic patients (both AA and PNH) showed in all cases high level of redundancy, with one or two sequences accounting for more than 50% of the entire Vβ-specific repertoire. Ten clonotypes reflecting major patient-specific clones were identified; in some cases, additional highly homologous clonotypes were found. All major clonotypic sequences were absent from normal controls, consistent with a putative pathogenic role for these clonal T cells. Inter-patient comparison of CDR3-sequences documented that all clonotypes were unique and patient-specific even if some recurrent motifs were seen. In 2 patients with similar HLA-background (3 out 4 identical class I antigens), clonotypes were different for just 3 amino acidic residues (98% homology within the total TCR-β chain), strongly suggesting a public/semi-public HLA-restricted immune response. A longitudinal analysis was possible in 4 AA patients treated with an anti-thymocyte globulin (ATG)-based immunosuppressive regimen: in all cases, great concordance between clonotype prevalence and blood counts was observed. The first patient, presented as moderate AA, showed a progressive increase of the pathogenic clonotype in concurrence with disease progression up to a severe AA, followed by significant decrease after successful immunosuppression. In 2 more patients, clonotype frequency decreased after an effective treatment, but 2 years later was again rising up, in the presence of clinical relapses. The last patient had no beneficial from immunosuppressive treatment, and showed no reduction of the pathogenic clone. In conclusion, dominant pathogenic T-cell responses are present in most AA and PNH patients, strongly suggesting an underlying common immune pathophysiology. Moreover, the homology between some patient-specific clonotype (mostly in presence of similar HLA-background) is suggestive of a public/semipublic immune response, likely driven by common antigens. Finally, the observation that the size of the pathogenic clone correlates with disease status leads to relevant clinical application, using longitudinal monitoring of pathogenic T cells to inform therapeutic decision in individual patients, such as additional needing or early tapering of immunosuppression.

Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder of hematopoiesis characterized by hemolytic anemia, thrombosis and bone marrow failure. In PNH a somatic mutation of the PIG-A gene results in deficiency of all proteins anchored by the glycosylphosphatidylinositol (GPI) on the membrane of the mutated hematopoietic stem cell (HSC) and in its progeny. The close association between PNH and idiopathic aplastic anemia, and numerous other pieces of evidence support the hypothesis that auto-reactive T cells might be at the heart of the pathogenesis of PNH. Specifically, these T cells might damage selectively normal HSC, whereas PNH HSC survive and expand because they escape the attack. Our recent observation of a unique patient with PNH and with a large granular lymphocyte (LGL) leukemia has strongly suggested the possibility that this clonal expansion of T cells, which have a CD8+ CD57+ phenotype, could be responsible for the damage to normal HSC in this patient. For this reason we have measured systematically the percentage of the CD8+CD57+ T cells in the peripheral blood of 12 PNH patients. In this series the proportion of this cell population was quite variable (8.4±6.8; range: 0.8-22.3%) and was very similar to that found in 18 healthy individuals (6.5±5.2; range: 0.9-21.2; p>0.5). Next, we investigated the molecular features of these cells. Sorted CD8+CD57+ T cells were characterized with respect to the size distribution of the complementarity-determining region 3 (CDR3) of the T-cell receptor (TCR) variable β (Vβ) chain genes. In healthy controls this analysis yields a ladder of normally distributed bands of different sizes. By contrast, in all 12 PNH patients this analysis yielded a markedly non-random (oligoclonal) pattern; and in each patient some clones were predominant. In three patients we were able to analyze follow-up samples and after 6 months or more the oligoclonal pattern was persistent. Sequencing of the TCR-Vβ chain genes in the expand-
ed clones is currently ongoing; and it may give some clue about the identity the target molecules of these clones. These observations strongly support the hypothesis that the CD8+ T cells population is involved in the pathogenesis of PNH.

**PO298**

**EXPANSION OF CD3+ TCR+ T LYMPHOCYTES EXPRESSING MEMBERS OF INHIBITORY RECEPTOR SUPERFAMILY (IRS) IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS**

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Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal disorder of the hematopoietic stem cell (HSC); characterized by an acquired somatic mutation in the PIg-A gene: this results in a deficiency on the cell membrane of all proteins anchored by the glycosphingolidinositol (GPI). PNH is closely associated to Idiopathic Aplastic Anemia. This fact, and our recent finding of increased frequency of expanded T-cell clones in patients with PNH (Karadimitris, Blood 2000), suggest that in PNH auto-reactive T cells that cause the selective destruction of normal HSC, whereas PNH HSC escape this T-cell-mediated attack, survive and expand. The identity of the auto-reactive T cells and of their target remains unknown. Recent studies have identified a subset peripheral blood T lymphocytes that have on their surface molecules belonging to the Inhibitory Receptor Superfamily (IRS). These surface molecules may belong either to the group of Killer Ig-like Inhibitory Receptors (KIR; e.g. CD158a, CD158b, p50.3), or to the group of C-Lectin type Inhibitory Receptors (CLR; e.g. the NGK2/CD94 complex). This set of T lymphocytes (CD3/TCR+, IRS+) may include chronically stimulated memory cells, and they may be expected to expand in the course of viral infection or of autoimmune process. We have analyzed the expression and function of KIRs and CLIRs in peripheral blood of PNH patients. The proportion of KIR+ cells within T(3D3+) lymphocytes was consistently higher in PNH patients (7.84±7.46, n=10) than in healthy donors (3.26±2.00, n=22, p<0.01, Mann-Whitney test). The ratio between KIR+CD3+ and KIR+CD3- cells was 2.61±3.43 in PNH patients and it was 0.52±0.50 in healthy donors; this ratio was higher than one in 60% of 10 PNH patients and only in 13.6% of 22 healthy donors (v<0.02, Fisher's exact test). Similarly, the proportion of CLIR+ cells (CD94+) was also increased. Based on the expression of the GPI-linked CD59, the KIR+CD3+ cells did not belong to the PNH clone. In order to investigate the function of these cells, we have isolated 20 KIR+ T cell clones from 2 PNH patients. Fifty per cent of these clones responded to engagement with appropriate antibodies by induction of cytolytic activity and by secretion of IFN-γ and TNF-α, indicating that they belonged to the activating KIR subset. We conclude that (i) KIR+CD3+ T cells are increased in PNH, as they are in autoimmune diseases; (ii) about one half KIR+CD3+ T cell clones from PNH patients bear activating isoforms of KIR. These CD3+ KIR+ cells include cells with cytotoxic potential that may be involved in the pathogenesis of bone marrow failure in PNH.

**PO299**

**A NOVEL PROTEOMIC TOOL IN FANCONI ANAEMIA DIAGNOSIS AND CHARACTERIZATION**

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Fanconi anemia (FA) is a rare cancer-prone genetic disease characterized by congenital malformations, bone marrow failure and chromosomal instability. Usually the death occurs in the first life decades due to the consequences of the bone marrow failure. The diagnosis is based on the sensitivity of patients’ chromosomes to diepoxybutane (DEB). The genetic heterogeneity (at least seven different genes) makes the molecular diagnosis difficult or impossible unless the mutated gene is known by complementation group determination. Knowing the complementation group is useful also for genotype-phenotype correlation studies and mandatory for prenatal and pre-implantation diagnoses and for gene therapy. It has been recently clarified that, following an injury to DNA, five of the seven known FA proteins bind together to form a complex that catalyzes the monoubiquitination (Ub) of a sixth protein (FANCD2) that then colocalizes in nuclear foci with BRCA1. This finding allowed the development of a new diagnostic tool based on the analysis of FA proteins by immunoblotting, as the finding of Ub-FANCD2 gives us informations about the function of the upstream five proteins too. Moreover, in case of absence of a specific FA protein, we immediately can classify the patient as mutated in the corresponding gene. The presence of the Ub-FANCD2 is also useful as a confirmatory test in complementation analysis by retroviruses. In this way, using specific antibodies against the proteins FANCA, FANCIG and FANCD2, we
translocated t(8;17)(q22;p13) as the sole structural
sis performed on bone marrow revealed a balanced
leukemic evolution (AML M4); the cytogenetic analy-
score: intermediate 2). Eight months after there was
normal karyotype and bone marrow blasts 15% (IPSS
syndrome characterised by severe thrombocytopenia,
20 months after FLUIC she developed myelodysplastic
apy and obtained complete remission after 6 months;
bine-Idarubicin-Ciclophosphamide (FLUIC) chemother-
karyotype; she was treated with 6 cycles of Fludara-
diagnosis; the cytogenetic analysis showed normal
hospital because of low grade non Hodgkin Lymphoma
patient, a 62 years old female, was admitted to our
we report a rare case of t(8;17)(q22;p13) in a sAML. The
8, 9, 12, 17, 21 and the chromosome 11q23 band. Here,
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MPO+, CD33 +, CD15 +, CD36 +, CD4 +, CD13 +, CD11c +,
CD34 +, CD14 +, CD11b - and CD117 -. The patient under-
got to Fludarabine, Cytarabine, Daunoxome (FLAD)
regimen obtaining complete remission, but died during
consolidation for pneumonia. To investigate a possible
p53 involvement, we performed fluorescence in situ
hybridization (FISH) analysis on metaphase cells with
p53 probe (Vysis Inc.): p53 gene was retained on 17p-
derivative chromosome; then using BAC mapping on
17p13, the breakpoint was revealed between 48B14
and 961a15, telomeric to p53; FISH with PAC 667k14,
revealed PAC involvement. Finally, FISH with probes
mapping on 8q showed the breakpoint between ETO
and C-MYC (studies ongoing). The translocation
t(8;17)(q22;p13) is extremely rare in AML: searching in
the Mitelman’s Catalog only two other cases of de novo
AML were found and the translocation was associated
with complex karyotype. This is the first case with
t(8;17)(q22;p13) as the sole anomaly and with molec-
lar characterization.

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MOLECULAR CYTOGENETIC CHARACTERIZATION OF
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Acute myeloid leukemia (SAML) secondary to
chemotherapy and/or radiation therapy for other
malignant conditions have emerged as important enti-
ties; SAML are often preceded by a myelodysplastic dis-
ease and associated in 75-100% of cases with high
incidence of cytogenetic abnormalities involving chro-
mosome 5 and 7 and, less frequently, chromosome 3,
8, 9, 12, 17, 21 and the chromosome 11q23 band. Here,
we report a rare case of t(8;17)(q22;p13) in a SAML. The
patient, a 62 years old female, was admitted to our
hospital because of low grade non Hodgkin Lymphoma
diagnosis; the cytogenetic analysis showed normal karyotype; she was treated with 6 cycles of Fludara-
bine-Idarubicin-Ciclophosphamide (FLUIC) chemother-
yapy and obtained complete remission after 6 months;
20 months after FLUIC she developed myelodysplastic
syndrome characterised by severe thrombocytopenia,
normal karyotype and bone marrow blasts 15% (IPSS score: intermediate 2). Eight months after there was
leukemic evolution (AML M4); the cytogenetic analy-
is performed on bone marrow revealed a balanced
translocation t(8;17)(q22;p13) as the sole structural
anomaly in all metaphases analyzed; the blasts were
MPO+, CD33+, CD15+, CD36+, CD4+, CD13+, CD11c+, CD34+, CD14+, CD11b- and CD117-. The patient under-
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lar characterization.

CHROMOSOME INSTABILITY IS ASSOCIATED WITH THE SEVERITY OF
COOLEY DISEASE
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Since the end of the 50’s, the evidence of erythrob-
lasts in mitotic phase with marked signs of structural
and functional abnormalities of karyocynetics in
peripheral blood and bone marrow of patients with
Cooley disease induced hematologists to search for
cytogenetic alterations. Besides aneuploidy to various
chromosomes, frequent breaks in chromatids in perih-
eral blood without prevalence for a particular type of
chromosome were detected. We evaluated the associa-
tion between the prevalence of such aberrations with
the severity of Cooley’s disease in 9 patients. All were
transfusion-dependent and were treated with subcu-
taneous chelation therapy. Median number of monthly
transfusion was 2 (range 1-3). Serum ferritin levels
varied from 800 to 3000 ng/mL. Chromosome instabil-
ity was estimated with quantative (number of breaks
per single metaphase and per 100 cells) and qualitative
(chromatid and chromosome breaks) methods. Five of
the 9 patients were identified as having a severe clin-
ic picture. Mean chromatid gaps was 14.8% (range 5-
24) and mean chromosome gaps was 3.4% (range 1-8).
Chromatid breaks were mean 2.1% (range 1-4) and
chromosome breaks were 1.1% (0-4). Severity of Co-
oley’s disease was inversely associated with chromatid
(gaps = 0.007) and associated with chromosome gaps
(gaps = 0.02). In fact, the 5 patients with a severe clinical
picture had significantly lower parentage of chro-
Telomeres are repeated DNA sequences that play a critical role in the maintenance of chromosomal stability in both healthy and cancer cells. Unlike many other cell subtypes, normal B-cells have highly variable levels of telomerase activity and TL according to their differentiation phase. In particular, a characteristic pattern of longer telomeres has been observed in GC-derived cells compared to GC-unexperienced subtypes. Aim of this study was to investigate TL in a large panel of MBCLD to verify whether it reflects TL of their normal counterpart or whether telomeres are uniformly short as observed in most non-lymphoid tumors due to progressive telomere erosion during tumorigenesis. To address this, 110 samples from patients with MBCLD containing at least 80% of tumor cells were evaluated. Our panel included 24 follicular lymphomas (FL), 14 diffuse large cell lymphomas (DLCL), 4 Burkitt’s lymphomas (BL), 9 marginal zone lymphomas (MZL), 9 mantle-cell lymphomas (MCL), 32 chronic lymphocytic leukemias (CLL) and 18 multiple myelomas (MM). Median age of patients did not significantly differ among various subtypes. TL analysis was performed by Southern blot using a chemiluminescence-based assay. TL was calculated using the Kodak Digital Science TM 1D Image Analysis Software. In our patient sample, median TL was 6060 bp (range 1896-14339 bp). TL in tumor samples did not correlate with patient age, as opposed to what has been observed in a control panel obtained from healthy subjects. FL, BL and DLCL had longer telomeres compared to other lymphoma subtypes (median 7447 range 5185-14339). CLL and MCL had the shortest telomeres (median 4120 range 1896-8961). MZL and MM had an intermediate TL. Analysis of VH somatic mutations has been carried out in order to discriminate MBCLD arising from GC-unexperienced or GC-experienced lymphoid cells. Telomeres are significantly longer in MBCLD arising from GC-experienced cells (median 6339 range 3360-14339) compared to GC-unexperienced subtypes (median 3707 range 1896-7383) (p<0.00001). We are currently assessing the presence of ongoing somatic mutations in order to differentiate tumors arising from the GC from those with a post-GC mutational pattern. In conclusion, our results indicate the following: i) TL in MBCLD is highly heterogeneous as opposed to most non-lymphoid tumors; ii) TL is effectively preserved during lymphomagenesis in GC-experienced cells, iii) due to particularly short telomeres MCL and unmutated CLL probably are the best targets among MBCLD for therapeutic intervention using the novel telomerase inhibitors which are currently undergoing pre-clinical evaluation as anti-cancer drugs.
CD34+ cell transduction rate was 26.1±7%, purity of normal donors and solid cancer patients (n=6). Mean of the respective untransduced counterpart, and transduced. We applied the transduction and selection in our transduction conditions, no CD138+ cell was transduced. We attempted to selectively eliminate myeloma cells from MPB grafts, by transducing CD34+ enriched cells with a Mo-MLV retroviral vector (BM-L-1) carrying the deltaNGFR gene, and by selecting transduced cells at the end of procedure. We designed patient-specific primers, based on the myeloma clonal IgH rearrangement, to assess the effectiveness of purging in each cell fraction at the beginning and at the end (deltaNGFR and NGFR+) of transduction and selection procedure. Our transduction protocol includes a 24-hour prestimulation of CD34+ cells with cytokines (SCF, TPO, FLT3-L at 50 ng/mL), one overnight transduction with BM-L-1 vector, and the immunoselection of transduced cells by an anti-deltaNGFR antibody 48 hours after transduction. We first optimized the procedure with CD34+ cells and deltaNGFR+/CD34+ selected cells was 29.7% of initial CD34+ cell number. We scaled-up the transduction procedure, reaching comparable results. Selected deltaNGFR+/CD34+ cells maintained an LTC-IC potential that was analogous to that of the respective untransduced counterpart, and transplanted in BM/Thymus of 7 SCID-hu mice gave rise to 52.2±15% CD45+, 49.5±23% CD19+, 57.1±15% CD4+, and 54.5±15% CD8+ bone marrow engraftment. In parallel experiments, we assessed the possibility of infection by retroviral vectors of selected CD138+ MM cells: in our transduction conditions, no CD138+ cell was transduced. We applied the transduction and selection procedure to MPB CD34+ cells obtained from 20 (small scale: 18 pt, large scale: 2 pt) aphereses of MM patients. Rate of CD34+ cell transduction was 28.9±11.6%, purity of transduced cells after deltaNGFR selection was 92.5±5%, and mean number of deltaNGFR/CD34+ selected cells was 29.7% of initial CD34+ cell number. We examined at different time points the presence of residual myeloma cells, with patient-specific PCR analyses. Ten out of 20 experiments were evaluable: 3 samples were PCR-negative for MM cells after CD34+ cell selection, 7 were positive in the CD34+ fraction: 4 of them lost myeloma contaminants during the culture, the other 3 lost the marker preferentially in the δNGFR+ fraction. We performed in 3 samples a limiting dilution assay, showing a 1-2 logs myeloma cells purging with culture procedure, and a further 0.5 log tumor elimination after δNGFR+ selection. We conclude that gene marking can be a feasible and efficient tool to confer a therapeutic benefit to MM patients. We noticed that transduction culture conditions itself can contribute to the purging; moreover, we showed a preferential myeloma purging in marked and selected cells.
involved in the skin and the liver was observed. The administration of multiple doses of ganciclovir (10 mg/kg/day), in the absence of immunosuppressive drugs, quickly resulted in the complete resolution of all clinical and biochemical signs of GvHD. In conclusion, Tk-DLI at a dose of 1×10^6/kg represents a promising tool for promoting immune reconstitution after haplo-SCT, while providing an effective and selective treatment for GvHD in the context of haploidentical SCT. A prospective multicentre European study is ongoing.

**PO305**

**RETROVIRUS-MEDIATED INTERLEUKIN-12 EXPRESSION IN ACUTE MYELOID LEUKEMIA-DERIVED DENDRITIC CELLS FOR ANTI-TUMOR IMMUNOTHERAPY**


“Institute of Hematology and Medical Oncology “L. & A. Seràgnoli”, University of Bologna, Bologna; °Immunotherapy and Gene Therapy Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

IL-12 is a very potent antitumor cytokine but its use in vivo has been limited by systemic toxicity. Several studies have evaluated the therapeutic potential of locally delivered IL-12 by cytokine-gene transduced tumor cells. To investigate the capacity of IL-12-producing tumor vaccine to overcome the inhibitory effect of the leukemic microenvironment, we stably transduced a human erythroleukemia cell line K562 with genes encoding for hIL-12 (p35 and p40). In a cell culture system which prevents cell-cell contact, transduced and not transduced K562 cells were co-cultivated in the presence of PHA stimulated T cells. At the end of culture, responder cells were intracellularly stained using different combination of clinical grade cytokines and CI. Encouraging results have been described when FAB M4-5 acute myeloid leukemias (AML) were put in culture, but few successes have been reported when different AMF-LAB subtypes or acute lymphoblastic leukemias (ALL) were used. Moreover, time to obtain DCs differentiation has been usually referred between 7 and 14 days and cell death was invariably associated. Recently the use of calcium ionophore (CI) was reported as capable to induce DCs differentiation from 15 patients with chronic phase CML. Aims of this study are to define optimal culture conditions for a rapid and reproducible DCs differentiation from AML and ALL using different combination of clinical grade cytokines and CI. In vitro differentiation of DCs from leukemic cells was tested using freshly isolated blasts from 9 cases of B precursor ALL, 1 case of T-ALL, and 5 AML patients (FAB M1). Different culture conditions were tested, but the combination of Stem Span serum free medium (StemCell Technologies, Vancouver, BC) supplemented with CI (100 ng/mL), GM-CSF (100 ng/mL) and IL-4 (50 ng/mL) gave the best results. Surface expression of CD40, CD80, CD83, CD86, CD54, CD58, HLA ABC and DR was analyzed at day 0, +4 and + 7 of culture. Moreover, FITC dextran (MW 70000) uptake capacity, induction of T cell proliferation, and cytokines production on allogeneic T lymphocytes were analyzed at day +5. After 4 days we could document an effective dendritic cell differentiation in 8/10 ALL and 5/5 AML. Indeed, the expression of costimulatory molecules, was significantly increased in all AM and B precursor ALL cases, but not in the T-ALL sample. CD80 expression at day 4 increased to a median value of 32%

**PO306**

**INNOVATIVE AND RAPID METHOD TO GENERATE MATURE DENDRITIC CELLS FROM ACUTE LYMPHOID AND MYELOID LEUKEMIC CELLS**

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An effective T cell response against leukemic cells largely depends on the immunogenic properties of these latter. The in vitro generation of dendritic cells (DCs) from freshly isolated leukemic cells could represent a useful approach to address this problem. Encouraging results have been described when FAB M4-5 acute myeloid leukemias (AML) were put in culture, but few successes have been reported when different AMF-LAB subtypes or acute lymphoblastic leukemias (ALL) were used. Moreover, time to obtain DCs differentiation has been usually referred between 7 and 14 days and cell death was invariably associated. Recently the use of calcium ionophore (CI) was reported as capable to induce DCs differentiation from 15 patients with chronic phase CML. Aims of this study are to define optimal culture conditions for a rapid and reproducible DCs differentiation from AML and ALL using different combination of clinical grade cytokines and CI. In vitro differentiation of DCs from leukemic cells was tested using freshly isolated blasts from 9 cases of B precursor ALL, 1 case of T-ALL, and 5 AML patients (FAB M1). Different culture conditions were tested, but the combination of Stem Span serum free medium (StemCell Technologies, Vancouver, BC) supplemented with CI (100 ng/mL), GM-CSF (100 ng/mL) and IL-4 (50 ng/mL) gave the best results. Surface expression of CD40, CD80, CD83, CD86, CD54, CD58, HLA ABC and DR was analyzed at day 0, +4 and + 7 of culture. Moreover, FITC dextran (MW 70000) uptake capacity, induction of T cell proliferation, and cytokines production on allogeneic T lymphocytes were analyzed at day +5. After 4 days we could document an effective dendritic cell differentiation in 8/10 ALL and 5/5 AML. Indeed, the expression of costimulatory molecules, was significantly increased in all AM and B precursor ALL cases, but not in the T-ALL sample. CD80 expression at day 4 increased to a median value of 32%
PROGRESSION IN T CELLS: ROLE OF CHK2 AND CDC25A

A NEW PATHWAY FOR THE INHIBITION OF CELL CYCLE PROGRESSION IN T CELLS: ROLE OF CHK2 AND CDC25A


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T cells encountering antigens under blockade of B7:CD28 costimulatory pathways become unresponsive, incompetent to expand and differentiate upon further stimulation. The mechanism(s) underlying blockade of cell cycle progression in unresponsive T cells are still poorly understood. Although it is known that T cells stimulated under costimulatory blockade overexpress p27kip1, a well known inhibitor of cell cycle progression, recent data have demonstrated that the presence of p27 is not essential to the induction of unresponsiveness, suggesting that other pathways may be involved in the process. In this study we investigated whether the ATM-chk2-Cdc25A pathway, that has been recently shown to be involved in the blockade of cell cycle progression in a variety of mammalian cells, upon irradiation and/or starvation, has a role in the induction of T cell unresponsiveness. Unfractionated peripheral blood mononuclear cells were stimulated with PHA for 3-6 days in the presence or absence of human recombinant CTLA4-Ig at 10 mg/mL (kindly provided by Dr. Maccario, Pavia, Italy); the amount of p27kip1, chk2, Cdc25A and cdk2 proteins was assessed on the harvested cells by western blotting and immunoprecipitation methods and labeled with specific antibodies. As we have previously demonstrated, T cell proliferation at 6 days was inhibited by 70-90% in the presence of CTLA4-Ig, whereas no difference was observed at earlier time points (1-2 days), confirming that CTLA4-Ig inhibits T cell expansion. To test whether the lack of T cell proliferation depended on defective cell cycle progression, we measured the concentration and the enzymatic activity of cycline-dependent kinase 2 (cdk2), which is essential for the entry into the S phase of the cell cycle, at various time points following stimulation. Stimulation of T cells with PHA was associated with an early (1-2 days) upregulation of cdk2 concentration and activity, that further increased up to 6 days. Interestingly, while CTLA4-Ig did not affect cdk2 concentration and activity early after stimulation, at 6 days cdk2 concentration and activity were both strongly reduced in unresponsive T cells as compared to controls (n=3). As the activity of cdk2 is known to be inhibited by p27, while it is induced by Cdc25A, through dephosphorylation, we tested whether the reduction of cdk2 activity in anergic T cells depended on chk2-Cdc25A pathway. Indeed, at 6 days, anergic cells showed markedly higher levels of p27 as compared to controls, whereas levels of Cdc25A were strongly reduced (n=3), thus suggesting that both pathways contributed to reduced cdk2 activity in unresponsive T cells. Interestingly, levels of Cdc25A were comparable in the presence or absence of CTLA4-Ig at 1-2 days. Finally, we tested whether the downregulation of Cdc25A depended on chk2, that is known to phosphorylate Cdc25A so as to increase its degradation rate. Levels of chk2 were markedly increased in CTLA4-Ig-treated T cells at 6 days, whereas they were comparable to control cells early after stimulation (n=3). In conclusion, our data suggest that the blockade of T cell expansion by CTLA4-Ig is accompanied by upregulation of chk2, leading to downregulation of Cdc25A levels, which in turn prevents activation of cdk2 and progression of the T cell into the S phase of the cell cycle. As the chk2-Cdc25A pathway has been shown to be induced by cellular starvation, we hypothesize that its activation in unresponsive T cells may be related to their lack of T cell growth factors such as IL-2. Development of agents specifically targeting these molecules might help to devise novel strategies to manipulate immune responses in vivo.
GRANULOCYTE COLONY-STIMULATING FACTOR PROMOTES THE GENERATION OF TOLEROGENIC DENDRITIC CELLS THAT INDUCE REGULATORY T CELLS

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We have recently demonstrated that G-CSF promotes the generation of T regulatory (TREG) cells capable of releasing high amounts of TGF-β1 and IL-10. In this study, we investigated whether the immunomodulatory effects of G-CSF might be mediated by dendritic cells (DCs). To this end, DCs were differentiated from CD14+ monocytes in the presence of serum collected before (pre-G) and after clinical administration of G-CSF (post-G). Similar to incompletely matured or semi-mature DCs, DCs cultured with autologous post-G serum expressed high levels of costimulatory molecules and HLA-DR, and exhibited diminished IL-12p40 release. Post-G DCs promoted tolerance in naive allogeneic CD4+ T cells and orchestrated a TREG response, as shown by a peculiar T-cell cytokine secretion profile (IL-2lowIL-4low/-IL-5+IL-10+TGF-β1+), and by suppression of nonregulatory CD4+ T cells that was crucially dependent on TGF-β1 and IL-10. Importantly, post-G DCs were completely insensitive to maturation stimuli but efficiently presented nominal antigens to autologous CD4+ T cells. As shown by neutralization studies, IFN-α and IL-10 contained in autologous serum and produced by marrow stromal cells upon G-CSF treatment, synergistically inhibited IL-12p40 release and promoted the acquisition of regulatory features by post-G DCs. Furthermore, phenotypic and functional features of post-G DCs were completely replicated by the addition of exogenous IL-10 and IFN-α to post-G monocytes during the DC differentiation process. Collectively, we identified a novel mechanism of immune regulation effected by G-CSF that might be exploited therapeutically for tolerance induction in autoimmune disorders.

PROGNOSTIC SIGNIFICANCE OF UNUSUAL KARYOTYPE ABNORMALITIES IN ELDERLY (>60 Y) ACUTE MYELOID LEUKEMIA(AML). RETROSPECTIVE ANALYSIS OF 37 PATIENTS

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The response to intensive induction in elderly patients with AML is usually disappointing since 1) age per se represents a poor prognostic factor, 2) the occurrence of cytogenetic abnormalities, though rare, may play an unfavourable role. Between February 1998 and May 2003, at our Institution, in 37 consecutive elderly pts (16 females and 21 males, median age 72 y, range 61-85y), conventional G-band analysis was employed. Results: out of 37 pts 25 (54.8%) exhibited clonal abnormalities, 8 (37.1%) resulted to have a normal karyotype, finally in 4 cases cytogenetics failed because of no metaphases: cytogenetic results are detailed in the table below.

<table>
<thead>
<tr>
<th>Cytogenetic Abnormality</th>
<th>n° patient</th>
<th>Frequency</th>
<th>age</th>
</tr>
</thead>
<tbody>
<tr>
<td>+8</td>
<td>6</td>
<td>16.2%</td>
<td>70 (m.a.)*</td>
</tr>
<tr>
<td>-7/del(7q)</td>
<td>5</td>
<td>13.5%</td>
<td>72*</td>
</tr>
<tr>
<td>+15/del(15q)</td>
<td>4</td>
<td>10.8%</td>
<td>75*</td>
</tr>
<tr>
<td>Complex karyotypes</td>
<td>4</td>
<td>10.8%</td>
<td>69*</td>
</tr>
<tr>
<td>-5/del(5q)</td>
<td>3</td>
<td>08.1%</td>
<td>72*</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>1</td>
<td>02.7%</td>
<td>62</td>
</tr>
<tr>
<td>t(9;11)(q22;q23)</td>
<td>1</td>
<td>02.7%</td>
<td>66</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>1</td>
<td>02.7%</td>
<td>65</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>6</td>
<td>22.7%</td>
<td>60 (m.a.)</td>
</tr>
<tr>
<td>No metaphases</td>
<td>4</td>
<td>10.8%</td>
<td>60 (m.a.)</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>100%</td>
<td>72 (m.a.)</td>
</tr>
</tbody>
</table>

*m.a.: median age.

As to induction, 18 pts were considered eligible for an intensive treatment: such as DNR+ARA-C(3+7) schedule (12), Gimema LAM 99 (4), and FLA-G regimen (2), respectively. The remaining 21 cases, which median age was 78, were treated with palliative (11), or supportive therapy, finally 2 patient died before treatment onset. As to response treatment 9 (50%) out the 18 patients treated with intensive regimen achieved CR, 1 PR; thus overall response rate (CR+PR) was 55,5%%; 5 were refractory, and 3 died during induction. In all CR cases cytogenetics at CR time was found to be normal. None of the patients treated with palliative approach achieved response and died. Complex karyotype abnormalities are usually associated with poor treatment response, while in our series 3/4 (75%) achieved CR, and only 4 of the 8 (50%) with normal cytogenetics were CRs. The high incidence (10.8%) of chromosome 13...
abnormalities in our series does seem to contrast with findings in other experiences, referring an incidence of 1%; this event may be related to the increased median age (75 y) and male sex prevalence. In elderly AML a correct assessment of cytogenetics at diagnosis and during treatment phases should be considered mandatory, since it can allow to detect abnormalities, up to now, defined rare as well as to define their role in influencing treatment response and disease outcome.

**PO310**
**CHARACTERIZATION OF A NEW CASE OF T(5;17)(Q35;Q21) VARIANT ACUTE PROMYELOCYTIC LEUKEMIA**


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Acute promyelocytic leukemia (APL) almost always involves a chromosomal translocation t(15;17) (q22;q21), that results in the fusion of the retinoic acid receptor alpha; (RARalpha) gene with the transcription factor gene PM L. Rare variant APL translocations have been described, involving translocations fusing RARalpha to partner genes other than PML. These translocations include t(11;17)(q23;q21), t(11;17)(q13;q21), t(5;17)(q35;q21) and dup(17)(q21.3q23), whereby RARalpha is fused to the PLZF, NuMA, NPM and STAT5b genes respectively. The t(5;17)(q35;q21) variant is a very rare abnormality; a total of four such cases have been identified. All these cases were pediatric (age: 2,5-12 years): 2 males and 2 females. Usually the leukemic promyelocytes exhibited hypergranular cytoplasm and absence of Auer rods. In all the cases RT-PCR demonstrated expression of NPM-RARalpha, while a reciprocal RARalpha-NPM fusion was described in three patients. Although, in the original report two expressed NPM-RARalpha isoforms have been found, presumably as a result of splicing, the three following case reports indicated expression of only the shorter NPM-RARalpha transcript. It seems there is evidence to indicate that the t(5;17) blasts can respond in vitro to the differentiating effects of ATRA, and in the one evaluable case treated with ATRA, the patient attained a remission. Here, we describe a further case of t(5;17) (q35;q21). The patient, a 29 year old man, presented with granulocytic sarcoma. A complete blood count revealed leukocyte count of 2.9x10^9/L, with a white cell differential of 78% neutrophils, 11% lymphocytes, 1% monocytes and 1% metamyelocytes. Haemoglobin was 8.3 g/dL and platelets were 101x10^9/L. A bone marrow aspiration showed a hypercellular marrow with 90% promyelocytes; Auer rods were not detected. A diagnosis of acute myelogenous leukemia subtype M3 has been made. The karyotype was: 46,XY,t(5;17) (q35;q21) (23/27)/46,XY (4/27). The involvement of RARalpha gene was confirmed by FISH analysis. RT-PCR demonstrated expression of NPM-RARalpha and RARalpha-NPM. Moreover, we identified two different isoforms of NPM-RARalpha: one is 94 nucleotides shorter than the other one. The patient was treated initially with idarubicin, cytospine-arabinoside and all-trans retinoic acid. Morphological and cytogenetic remission were obtained. Molecular remission was reached after other three cycle of therapy, including idarubicin, mithoxantrone, etoposide, 6-thioguanine and all-trans retinoic acid.

This work was supported by grants from: Ateneo 60%, Fondazione del Monte di Ravenna e di Bologna.
It becomes clear from above that the entire array of B lymphocyte disorders (autoimmunity, MGUS, overt neoplasia) is present in AAE. We report our experience of 23 AAE patients followed up to 25 years, and reviewed the data of the literature. The patients have been followed for a median of 8 years. C1-Inhibitor activity was measured with chromogenic assay. Autoantibodies to C1-Inhibitor were detected by ELISA. Median age at onset of angioedema was 57 years, range 39–75. All patients had C1-Inhibitor function and C4 antigen below 50% of normal. C1q was reduced in 17 patients. Autoantibodies to C1-Inhibitor were present in 17 patients. Long-term prophylaxis of attacks with danazol was effective in 2 of 6 patients, and tranexamic acid in 12 of 13 patients. Therapy with C1-Inhibitor plasma concentrate was necessary in 12 patients: 9 had rapid positive response, 3 became progressively resistant. Associated diseases at the last follow up were: 3 non-Hodgkin lymphomas, 1 chronic lymphocytic leukemia, 1 breast cancer, in 13 monoclonal gamopathies of uncertain significance (MGUS). In 4 patients no pathologic condition could be demonstrated. AAE patients present higher risk for B cell malignancies than general population, with regard to the presence of MGUS, none of our 13 patients with MGUS in a median follow up of 8 years presented disease progression to myeloma suggesting that their risk of malignancy is not higher than in other patient with MGUS. The presence of autoantibodies to C1-INH do not exclude the possibility of developing B cell malignancies. Anti-fibrinolytic agents are more effective than attenuated androgens in long-term prophylaxis: AAE patients can be resistant to replacement therapy with C1-inhibitor plasma concentrate.
A CASE REPORT
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Natural killer cells are a distinct no-T no-B lineage of lymphocytes. Malignant hematolymphoid disorders arising from NK cells, have become recognized in past decade. Phenotypically different disease can be distinguished with aggressiveness and response to treatment different. In our Division we have observed, in the last 12 months, 3 cases of NK pathology with different phenotypic characteristics and aggressiveness, they have been treated with different therapies. CASE 1: 81 year-old man has received in July 2001 diagnosis of refractory anemia and expansion of CD16⁺/CD56⁺ population. Epoetin and steroid treatment failed and increasing transfusion requirement. In October 2001, a new medullary aspiration has shown a PRCA and has confirmed NK expansion. The molecular biology, the viral and tumor examinations are results negative. In January 2002 has been started unsuccessfully therapy with cyclosporine. In June 2002, transfusion requirement was of around 3 bags of blood every week, worsens the
pancitopenia and persist NK expansion. In July 2002 has started therapy with low doses of cyclophosphamide (50 mg/day). From August 2002 to February 2003 the patient has never been transfused and the hematological values were normal. Relapsed in March 2003 has been necessary transfusion therapy (around one bag every two weeks) and increase of cyclophosphamide therapy (100 mg/day). The patient has died in May 2003 for pulmonary infection.

Case 2: 36 year-old man, in July 2002 has received diagnosis of NK acute myeloid leukemia (phenotype: CD7+; CD56+; CD34+; CD33+; CD117+; cyCD3+) with cutaneous interest and has started DCE induction; the aplasia has been complicated by a pulmonary aspergillosis and has succeeded in having complete remission. In December 2002 has been documented relapse of the leukemic disease and the patient has been treated with MEC scheme with which has succeeded to have new complete remission, consolidated in February 2003 by new MEC therapy. In May 2003 new relapse of leukemia disease and the patient has started lifesaving therapy waiting for a possible transplantation. CASE 3: 73 year-old man, in September 2002 has received diagnosis of NK lymphoblastic lymphoma with cutaneous and medullary interest (phenotype: CD56+; CD4+; CD2+, CD5+; HLA-DR+; CD36+; CD33+), has started CHOP therapy × 6 cycles succeeding in finding complete remission. In March 2003 medullary relapse of lymphoproliferative disease has been documented (60% of cells CD56+; CD4+) and for bad general conditions have begun CVP therapy from heart disease. In conclusion, NK diseases have various biological characteristics that often require aggressive therapies but with disappointing results. Transplant strategies are suitable in this type of pathology and immunological therapies might be indicated for the control of minimal residual disease (e.g. Campath-1H; αIFN).

PU005
DEFECTIVE CD1c EXPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA
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Using oligonucleotide microarray analysis to profile the expression of chronic lymphocytic leukemia (CLL) B cells, Zheng et al. (Leukemia, 16:2429, 2002) have recently shown that CLL cells significantly down-regulate transcripts from CD1c genes encoding proteins known to be involved in innate and adaptive immunity. The available data concerning CD1c antigen expression in B-cells from chronic lymphoproliferative diseases (CLD) are few and not recent. Since 1990, and as part of our routine diagnostic panel, we have evaluated by means of flow cytometry CD1c expression in peripheral blood lymphocytes (PBL) from 735 patients with CLD and in lymph node cell suspensions (LNC) from 173 patients with non-Hodgkin lymphoma (NHL). The 735 cases of CLD were diagnostically defined as follows: 505 cases of classic B-CLL, 78 cases of variant-CLL (sIg+CD5+/CD23 variable/FISH t(11;14) negative), 31 cases of leukemic forms of mantle cell lymphoma (sIg+CD5+/CD23 variable/FISH t(11;14) positive) and 121 cases of CD5, CD10 and CD103 negative forms. The histologic diagnoses of the 173 NHL cases were 28 lymphocytic NHLs (SLL), 17 mantle cell lymphomas (MCL), 24 lymphoplasmacytic/immunocytomas (LP-IC), 21 marginal zone lymphomas (MZ-NHL), 46 follicular lymphomas (Fo-NHL) and 37 diffuse large-cell lymphomas (LCL-NHL). The PBL analyses showed that the frequency of CD1c expression was lowest in the patients with B-CLL (95/505 positive cases, 18.8%; p <0.0001); furthermore, the positive cases frequently (61%) showed partial CD19/CD1c coexpression (>20% and <60% of B-cells; degree of reactivity: 1). In the other forms of CLD, CD1c expression was very common (more than 80% of cases) and involved the majority of B cells (degree of reactivity: 2). A similar pattern was observed in the patients with low-grade NHLs: only 11/28 cases of SLL (39%) showed CD1c positivity (prevalent degree of reactivity: 1); in the other low-grade histotypes, CD1c expression (prevalent degree of reactivity: 2) was found in 72-85% of the cases. Interestingly, a low percentage of positive cases was also observed in LCL-NHL (14/37 casi, 37.8%), although the degree of CD1c reactivity was 2. An overall comparative analysis of the relationships between the expression of CD1c and that of the other tested CDs revealed an inverse correlation (Pearson coefficient = -0.5) with CD43 and a direct correlation with CD79b and CD49d (Pearson coefficient = 0.5). No relationship was found with CD38 expression in B-CLL. The defective expression of CD1c antigen in CLL/SLL patients is not only relevant diagnostically, but also biologically: the possible meaning of its down-regulation needs to be clarified by means of further biological studies.
Conventional therapy for chronic HCV is actually based on the employment of a combination therapy with peg-interferon-α (PEG-IFN) plus ribavirin (RBV). There are reports that a combination of interferon-α with ribavirin is efficacious in the treatment of patients with HCV positive mixed cryoglobulinemia (MC), while there are no controlled studies of a therapy with PEG-IFN plus RBV in patients with HCV positive MC. Aim of the Study: The aim of this study was to evaluate the efficacy of and the tolerance of therapy with PEG-IFN plus RBV in patients with chronic hepatitis C related MC. Materials and Methods. Fifteen consecutive patients with MC were recruited into the study (8 F/7 M, median age 52±8 yrs). Of these, 80% had type II MC and 20% had type III MC with a median cryocrit of 4.1%. Severe chronic hepatitis was present in 11 cases (73.3%), while mild chronic hepatitis was observed in 4 cases (26.7%). All cases were HCV-RNA positive, genotype 1 (53%), or non-1 genotypes (47%). The 15 patients were treated with peg-interferon α2b at a dose of 1 μg/kg per week plus Ribavirin 800 or 1000 mg per day for 24 weeks and after followed for an additional 24 weeks. Only medium to low-dose steroids were allowed, already administrated at the time of recruitment. Results: At the end of therapy, we observed a reduction of the mean cryocrit level (from 4.12±0.9% to 2.0±0.6%, p<0.02), of the mean ALT level (from 141.9±51 to 36±6, U/L p<0.05), and of the purpura score (from 2.0±0.6 to 0.4±0 p<0.05). Moreover, we observed the complete virologic response in 11 cases (73.3%). Virological response was higher in patients with genotype non-1 (5 cases) than in patients with genotype 1 (2 cases). In one patient with glomerulonephritis, a reduction in proteinuria was observed but at the completion of therapy, the proteinuria returned to pretreatment levels. Four cases (26.6%) obtained no response. Treatment was well tolerated. One case discontinued therapy to 12 weeks for autoimmune thyroiditis. At the end of follow up 7 patients (46.6%) had sustained virological, biochemical and a symptomatic vasculitis response, without detectable cryoglobulins in serum. In conclusion, in patients with MC, PEG-IFN plus RBV therapy was more effective than combination standard therapy (IFN+RBV). Clinical and immunological response seems to be correlated with the eradication of HCV. PEG-IFN plus RBV may represent a safe and effective alternative to standard immunosuppression in MC.
without any hematopoietic growth factor therapy or support.

**PU008**

**CLINICAL AND MOLECULAR COMPLETE REMISSION IN A CASE OF VARIANT HAIRY CELL LEUKEMIA TREATED WITH DHAP FOLLOWED BY HIGH-DOSE CHEMOTHERAPY PLUS RITUXIMAB**

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Variant hairy cell leukemia (v-HCL) is a rare chronic B-cell lymphoproliferative disorder whose aggressive clinical course leads to short survival times. The conventional treatment options for HCL (purine analogues, α-interferon, splenectomy) are often less effective in v-HCL. We here describe a case of a 53-year-old man affected by v-HCL. He presented conspicuous splenomegaly (10 centimetres from the costal arch) and slight hepatomegaly. There was no lymph adenopathy, hemoglobin was normal and blood neutrophils were 3.1×10^9/L. The patients was sequentially treated with 2'-deoxycoformycin, α-interferon, splenectomy and aggressively relapsed after 12 months with deep and superficial lymphadenopathies (bulky in the axillae), leukocytosis (WBC 70.0×10^9/L) due to the massive presence of circulating hairy cells, and diffuse bone marrow involvement. After an unsuccessful approach with an antracycline-including regimen, the patient was efficaciously treated with a DHAP-like protocol. With the aim to collect purified PBSCs, the fifth DHAP cycle was administered with G-CSF, followed by an in vivo purging with two doses of Rituximab. The presence of residual hairy cells in the peripheral blood stem cells (PBSCs) was excluded by assessing DNA samples from the leukapheresis products using semi-nested PCR amplification of the specific clonal rearrangement of IgH genes. One month later, the patient underwent high-dose chemotherapy (melphalan + thiotepa) followed by autologous PBSCs infusion and two more rituximab doses on days +30 and +37. He is still in complete clinical and molecular remission after 12 months of follow-up. This case offers a number points of interest. A conventional regimen (DHAP), which has previously been used as a pre-high-dose therapy salvage treatment in relapsed aggressive NHL and, more recently, in a successful schedule for the treatment of mantle cell lymphoma, proved to be extremely efficacious in this case of advanced v-HCL. The sequential use of anti-CD20 monoclonal antibody allowed us to collect molecularly purified PBSCs, thus extending the therapeutic role of rituximab to v-HCL, after its previous successful use in typical HCL. Furthermore, the good results achieved with this sequential therapy are of particular interest because 2'-deoxycoformycin, α-interferon, splenectomy and an antracycline-containing regimen had been ineffectual in attaining a lasting response.

**PU009**

**THE ASSOCIATION IFOSFAMIDE-FLUDARABINE-RITUXIMAB AS A THERAPEUTIC OPTION IN ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA**

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Fludarabine is efficacious and safe in CLL patients refractory or relapsing after Chlorambucil. It has been shown that its effectiveness is enhanced by the association with alkylating agents, mainly represented by Cyclophosphamide, and monoclonal antibodies. Iphosfamide is an oxazophorin, analogue to Cyclophosphamide, whose efficacy has been demonstrated in the treatment of solid neoplasia and, in the hematologic setting, in NHL, but not in CLL. In order to assess the safety and efficacy of an Iphosfamide, Fludarabine and Rituximab containing regimen, we treated 9 CLL patients (M/F 5/4, IWCLL BII (3), CIII (1),C-IV (5), median age 66 yrs, range 54-69) affected by refractory or relapsing disease with an IFLU regimen (Iphosfamide 750-1000 g/m² days 1-3, Fludarabine 25 mg/m² days 1-3) in combination with Rituximab (Mabthera 375 mg/m², day 1). Initially we started with an IFLU regimen and added Rituximab from the fourth cycle (3 cases). Subsequently, due to the absence of adverse events, Rituximab was given together with IFLU from the first cycle. Subsequently, after the completion of the chemotherapy courses, Rituximab was administered on a monthly basis in the context of a manteinance schedule. A median of 2 R-IFLU cycles (range 1-4) were performed. Efficacy: four patients obtained PR (including a durable PR after only one cycle of therapy), one showed refractory disease, 3 are not evaluable (too early). Two patients are still in PR after 11 months of follow-up, while remission duration was 6 and 8 months in the other 2 (median duration of the response, 9 months). Safety: one patient died for infection (sepsis during aplasia). Other infections observed were a HBV reactivation (controlled by lamivudine therapy) and a fever of undetermined origin. WHO Grade 3-4 neutropenia occurred in 6 patients and thrombocytopenia...
of WHO grade 4 in one case. These preliminary data, obtained in a setting of patients heavily pretreated, show an ORR of 66% with an acceptable incidence of infective complications and suggest that a chemotherapy regimen combining Fludarabine with Iphosfamide and Rituximab may be a feasible and efficacious choice in patients affected by advanced CLL.

PU010
POSTREMISSIONAL RITUXIMAB ADMINISTRATION FOR THE TREATMENT OF POOR PROGNOSIS CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS
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On the basis of the safety and efficacy of Rituximab used as a single agent in CLL, we conducted a clinical study to determine the safety and efficacy of Rituximab given as consolidation therapy in 14 patients with advanced and previously treated CLL. All patients were responsive (CR: 1; PR: 11; nPR: 2) to a prior advanced line of treatment (median prior CHT schedules: 3) with a Fludarabine (Flu) combining therapy (FAND: 6 pts; Flu + CTX: 3 pts) or with alkylating agents (CB: 1pt; CHOP: 1 pt; HD-CTX: 2 pts). In 7 cases (50%), the leukemic cells had unmutated IgVH regions. The median number of residual CD5+/CD20+ lymphocytes was 127 mm3 (range 6-2580/mm3) in the Pb and 13.5% (range 1-40%) in the BM; 6 patients showed residual enlarged nodes (max. Ø 2.5 cm). Patients received 4 weekly doses of Rituximab (375 mg/m2) and were completely restaged 6 weeks later and thereafter every 3 months up to disease progression. Treatment was well tolerated. Mild infusion-related side effects were noted only in one patient at the time of the first Rituximab infusion. No infectious toxicity was observed. Following Rituximab administration, 10 of the 13 patients treated in PR achieved a clinical CR and the patient treated in clinical CR achieved a cytometric CR; 2 patients did not improve their response assessment and 1 showed a progressive nodal enlargement. Taken together, 11 patients (78%) showed a further reduction of their residual disease and 8 achieved a cytometric CR. The median rate of residual CD5+/CD20+ lymphocytes in the aphereses was 1, 1, 3 and 4%, respectively. During the follow-up, all patients showed a slow increase of PB and BM leukemic cells over time, with an overt clinical relapse (PB lymphocytes >4000/mm3 and/or nodal enlargement) in 6 cases after a median time of 9 months. A new Rituximab administration was offered to 3 patients. Two were treated in cytometric relapse after 9 and 18 months from the first administration and had a second cytometric remission (=12; +6 months) which was followed by a new cytometric relapse after 12 months in a patient who underwent a third Rituximab administration. The patient who received the second Rituximab administration while in nodal relapse showed no response. In conclusion, in this group of heavily pre-treated CLL patients the postremissional administration of Rituximab proved safe and effective in reducing residual disease in the majority of cases. The therapeutic benefit of Rituximab and the optimal schedule of Rituximab administration needs to be further investigated.

PU011
MONOCLONAL GAMMOPATHY DURING CMV INFECTION IN CLL TREATED WITH FLUDARABINE (FAMP) + CYCLOPHOSPHAMIDE (CTX) AND LOW DOSES OF MAB-CAMPATH
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The patient is 45 years old man; in march 2002 diagnosis of B-CLL was performed. At that time, hepatosplenomegaly was present in association with bilateral axillary lymphoadenopaties. WBC count was 260×10^9/L (lymphocytes 90%). Hb 11.5 g/dL, PLTS 136×10^9/L. Peripheral blood phenotype of monocuclear cells was: CD19 96%; CD5 95%; CD38 1.5%; CD3 2.3%; CD24 93%; FM C7 1.7%, k-chain 90%.Bone marrow biopsy showed a diffuse infiltration of CD20, CD5, Bcl-2 positive small lymphocytes. No HLA matched familial donor was found. From April to August 2002 he was submitted to 6 courses of FAMP 30 mg/ m^2 + CTX 300 mg/m^2 day 1-3. At evaluation of response, he obtained a normalization of peripheral blood cell count but the bone marrow biopsy showed a 80% of lymphocytes infiltration with a nodular pattern and persisting splenomegaly. In November 2002, Mab Campath 10 mg subcutaneous 3 times/week for 6 weeks was started. During treatment, antigenemia for cytomegalovirus (CMV) was persistently negative. During the last two weeks of therapy, the patient presented fever and, at the same time, a IgG k+κ monoclonal
gammopathy (1.4 g/dL) appeared. CMV antigenaemia was negative and after few days patient presented a recovery of fever so that therapy course was completed. Seven days after therapy discontinuation, the patient presented diarrhea and fever; CMV antigenaemia was negative but CMV was discovered in faeces by PCR. M-component was at the same time increased to 2 g/dL. Treatment with oral gancyclovir was started at dosage of 3 g/d for two weeks, with a complete recovery of clinical symptoms: after a week of anti-viral treatment, PCR-CMV in faeces resulted negative. We recorded a further increase in gammopathy with a maximum peak to 4 g/dL 15 days after CMV infection evidence. At the same time, a re-evaluation of disease was performed: peripheral blood cell count showed Hb 12 g/dL, Pts 143 × 10^9/L, WBC 2.6 × 10^9/L (N 66%, lym 19%). Peripheral blood immunophenotype of mononuclear cells was: CD3 43%, CD4 15%, CD8 29%, CD16 36%, CD19 21%, κ-chain 14). Bone marrow biopsy showed a 10% of small CD5+ lymphocytes. Spleen volume was reduced, but a mild splenomegaly persisted. In the following months, value of monoclonal component decreased, to 1 g/dL (May 2003).

PU012
RITUXIMAB TREATMENT FOR CLL PATIENTS IN MOLECULAR OR IMMUNOLOGIC RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION
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Between 1995 and 2002, 30 chronic lymphocytic leukemia (CLL) minimal residual disease patients (pts) with advanced disease were autografted at our Institution. Eighteen of 30 pts (60%) showed a clinical relapse after a median time of 31.5 months (range 2-79) from transplant and 16 underwent further treatment, with a median interval from relapse to treatment of 3.5 months (range 1-38). Various schedules of therapy were utilized and 14 pts achieved a response, which was complete in 1; only 2 patients were refractory to two lines of therapy. Twelve of 18 relapsed patients had achieved a molecular remission after autograft and in all these patients the reappearance of the molecular signal was predictive of a subsequent immunologic and clinical CLL relapse. To assess the value of early treatment in delaying clinical relapse, the anti-CD20 monoclonal antibody Rituximab was utilized in 4 patients in clinical complete remission who showed an immunologic or molecular recurrence. Of these 4 pts, 3 who presented an immunologic recurrence 6, 24 and 51 months after autograft, were treated 20, 5 and 15 months later, respectively; 1 pt, who showed a molecular relapse 24 months after transplant, received Rituximab 19 months later. Treatment schedule included a conventional dose of Rituximab (375 mg/m^2) once a week for 4 infusions. Only 1 pt experienced therapy-related toxicity, associated to the third dose, including fever, chills and articular pain, which required treatment interruption. Of the 3 pts treated in immunologic relapse, 1 did not respond, showing a clinical relapse after 11 months from Rituximab treatment and 2 pts obtained an immunologic remission. Of these 2 latter pts, 1 is persistently in immunologic remission 11 months after Rituximab and the other is in immunologic relapse 9 months after treatment, while remaining in clinical and hematologic remission. The patient treated in molecular relapse showed an immunologic relapse 5 months after anti-CD20 administration, persisting in clinical and hematologic remission 16 months later. Based on our limited experience and on literature data some considerations may be drawn: the administration of Rituximab in the setting of minimal residual disease in autografted CLL patients may induce objective responses, the sequential administration and higher doses of Rituximab need to be tested in this clinical situation and alternative modes of anti-CD20 utilization should be considered (e.g. early employment for in vivo purging, use as maintenance therapy after autograft).

PU013
HISTIOCYTIC NECROTIZING LYMPHADENITIS (KIKUCHI’S DISEASE): REPORT OF TWO CASES
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Kikuchi disease or histiocytic necrotizing lymphadenitis is a self limiting disorder, which usually affects young women and manifests clinically by cervical lymphadenopathy with or without fever. The aetiology remains undetermined, although a viral or autoimmune hypothesis has been suggested. The diagnosis is done only by histological examination of the lymph node biopsy, characterized by necrosis and histiocytic infiltration without neutrophils. Given the rarity of the disease, we report here the clinical and pathological description of two new cases, diagnosed at our Department during the last 3 years. First case: a 30-year-old woman was admitted to our hospital 3 years ago because of left cervical lymphadenopathy, mild fever, neck pain and night sweats. Blood cells count, biochemical assays, urinalysis were all in the normal range; neoplastic, auto-immunity markers and serological assays were negative; a computed tomography
scan of the neck, chest and abdomen shown only the enlargement of one lymph node (2 cm) of the left cervical region. A biopsy was performed, with diagnosis of histiocytic necrotizing lymphadenitis; the immunophenotype examination shown cells positive for CD3, CD2, CD4, CD8, CD7, peripherine, granzyme and myeloperoxidase; negative for EBV and TIA1. The clinical picture improved spontaneously and patient was discharged without any specific therapy. Six months later, an enlargement lymph node of cervical right region appeared, without any clinical symptoms and with a quick, spontaneous remission. One years later the patients suffered from a second relapse, at left cervical lymph nodes with neck pain, fever and night sweats, with a complete remission after few days. Second case: a 24-year-old woman was admitted to our hospital six months ago because of right cervical and bilateral axillary lymphoadenopathy, without clinical symptoms and with normal blood parameters; a computed tomography scan confirmed the presence of superficial lymphadenopathy only. The histological examination shows a picture of Kikuchi disease and the patient was discharged without any therapy. Until now, no relapse was observed in this case. Our limited experience confirms that Kikuchi disease is a very rare, benign lymphoproliferative disorder, diagnosed in particular in young women. In the first case, given the clinical symptoms and the course of the disease, with frequent relapses, a differential diagnosis with lymphoproliferative neoplastic disease must be considered.

PU014
DIRECT ANTIGLOBULIN TEST IN 120 PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA

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A positive antiglobulin test in patients with diagnosis of chronic lymphocytic leukemia is a relatively common event (10%), sometimes unexpected in patients with normal hemoglobin level. We studied 122 consecutive patients with CLL, 48 female and 74 male, with a median age of 68 (range 36-87 years), observed in the outpatient ward. They were studied in some detail for the presence of antibodies on red blood cells. DAT was performed by ORTHO Autovue system in our Immunohaematology and Transfusion Center. Eluate of DAT positive red cells was analysed in order to identify the subtype of the involved immunoglobulin. We found 10 subjects (9%), 4 female and 6 males, with positive DAT. This result was expected in patients who had a previous diagnosis of autoimmune hemolytic anemia (AHA) (Hb < 4 g/dl), and had already been treated by steroids and immunosuppression. The other 8 DAT positive patients had normal blood and reticulocyte counts. The immunohaematologic study showed in 9 cases the presence of panagglutinin IgG and in one patient positivity only for C3d. The subjects with positive IgG were studied for identification of subtype of the involved immunoglobulin by DiaMed-ED micro typing system and ID-Card DAT IgG1/IgG3: in 2 cases we found positivity for IgG1. The other samples were all negative for IgG1 and IgG3, thus presumably IgG2 or IgG4. In one patient with positive DAT without anemia and associated idiopathic thrombocytopenic purpura, we observed disappearance of DAT positivity during the treatment with anti-CD52 monoclonal antibodies (CAMPATH-1H) and reappearance three months after therapy. No correlation between positive DAT and lymphocyte immunophenotype emerged in our study. Our results confirm the presence of antibodies on red cells surface does not necessarily mean hyperhemolysis; however, a close monitoring of these patients is needed, since additional often unknown factors may suddenly cause massive red cell destruction. As for the treatment, steroids and immunosuppressant drugs (such as azathioprink and mofetil mycophenolate) may control hyperhemolysis, and the new monoclonal antibodies may reduce or even abolish autoantibody production.

PU015
PROGNOSTIC VALUE OF CD38 IN CLL

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The search for prognostic factors to identify subjects at risk of disease progression and thus eligible for intensive therapeutic programs in patients with Chronic Lymphocytic Leukaemia (CLL) is a main goal of biological and clinical studies. CD38 is a transmembrane protein with unidentified function, the prognostic significance of which is going to be verified. We studied 110 patients with typical CLL (CD5+, CD23+, with low expression of Smig) observed from January 2000 to February 2003. A peculiarity of our study was the detection system of CD38 expression used, based on the measurement of fluorescence intensity expression on the lymphocyte population (MIF), rather than on the evaluation of the percentage of positive cells. We stratified the patients into two groups: first group composed of patients showing low expression of CD38 (using 10 MIF as cut-off) and a second group composed of patients with expression of this molecule higher than 10 MIF. The search of possible differences of hematologic parameters (haemoglobin, white cell and platelet
counts) between the two groups, did not show any statistical correlation; by contrast the analysis of clinical parameters at diagnosis or during the follow-up showed interesting correlations. Patients with high CD38 had larger lymphoadenopathy, more severe epatomegaly, more frequent infective or autoimmune complications and higher need of treatment as compared to the low CD38 group. Fluorescence intensity expression remained stable during the follow-up, both in treated and not treated patients. Our results support the evidence that CD38 expression identifies at diagnosis patients with worse prognosis. The technique used is reproducible, sensitive, easy to be performed and less expensive compared to othermore sophisticated prognostic parameters.

Acute Lymphocytic Leukemias

**PU016**

**JH ANALYSIS SUGGESTS THAT THE ACQUISITION OF PH CHROMOSOME MAY BE A SECONDARY EVENT IN P190 POSITIVE B-ALL**


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The patient M.A. is a 49-years-old woman who came to our attention in July 2001 because of fever and fatigue. The immunophenotypic and cytochemical analysis allowed to establish a diagnosis of CD10+ B-ALL. The karyotype, performed on bone marrow cells, was 46,XX in 30% of metaphases and 44,XX,t(9;22)(q34;q11), -7, add 12,-14 in the remaining 70%. Following the Codox-M induction regimen the patient achieved a partial remission (PR). Then she was salvaged by FLAN regimen which produced a complete hematological and cytogenetic remission. On December 2001 she started a maintainance therapy with 600 mg daily imatinib mesylate and α-interferon (3 MU × 3 weekly). After 6 months, the patient relapsed. Cyto genetic analysis, performed on marrow cells, was normal. At diagnosis, by Jh amplification, a rearranged Jh clone was identified and always detected during follow up controls. Similarly, a clonal rearranged bcr/abl (e1a2) was identified and found in each analysis. Hemopoeisis resulted clonal by the use of the x-linked marker Humara at diagnosis, and became polyclonal during disease remission. The second patient, C.R., is a 36 years old female who was diagnosed as having CD10+ B-ALL in June 2002. The cyogenetic analysis displayed a complex karyotype with t(9;22) in all the studied metaphases. The molecular study revealed the presence of a clonal rearranged bcr/abl (e1a2) and a clonal Jh rearrangement. Humara gene analysis evidenced a clonal pattern. After a double induction with HYPER-CVAD regimen the patient achieved a complete hematological and cytogenetic remission. The molecular evaluation revealed the persistence of both clonal markers (Bcr-Abl and Jh). Humara test showed a polyclonal pattern. On September 2002 the patient underwent HLA matched sibling allogeneic transplant. At eight months from the transplant the patient is alive and disease free. The karyotype is normal, the Bcr-Abl traslocation is undetectable with RT-PCR and real-time PCR. The Jh gene amplification detects the persistence of the clonal rearranged band observed at diagnosis. The following Table 1 summarizes the reported findings.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>Bcr/Ab1</th>
<th>JH</th>
<th>HUMARA</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.A.</td>
<td>Ph+</td>
<td>pos</td>
<td>pos</td>
<td>clon</td>
</tr>
</tbody>
</table>
| CR (post induct.) | Ph−       | pos     | pos | policl.
| Post-BMT | n.a.      | n.a.    | reg | n.a.   |
| Relapse | Ph+       | pos     | n.a. | n.a.   |

The biological behaviour of these two patients allows us to make some speculations on the pathogenetic model of P190 positive B-ALL. According to the multi-step Knudson hypothesis of leukenogenesis two possible pathways might be designed. i) the Ph+ clone coexists at diagnosis with a Ph− clone. Imatinib therapy is able to control only the Ph+ clone leading to the progressive expansion of the Ph− leukemic cells. ii) the initial leukemic clone is Ph− and because of genetic instability it acquires the t(9;22) as secondary genetic lesion. The observation of a Ph− relapse in the first patient and the detection of only a clonal Jh rearrangement during CR, in the second, seem to be better explained by the second hypothesis.
We present a case of extensive livedo reticularis in the setting of T-cell acute lymphoblastic leukemia and discuss clinical features underlining the importance of histological examination. Case report. An 18-year-old man was admitted at our institution with fatigability, abdominal pain lasting for about 20 days. Previous medical history was negative. Clinical examination showed diffuse lymphadenomegaly, splenomegaly and a painful livedo reticularis-type skin rash of the trunk and inferior limbs. The peripheral white blood cells counts was 47,000 mm$^3$, hemoglobin 9.4 g/dL, platelets 98,000 mm$^3$. Bone marrow examination was consistent with acute lymphoblastic mature T-cell leukemia (immunophenotype: CD7+, CD2+, CD5+, CD8+, cCD3+, Tdt:neg). To better clarify the pathological nature of the cutaneous disease (leukemia-related lesion or coexistent dermatois) a cutaneous biopsy from legs was performed and it showed CD3$^+$ and CD5$^+$ cells diffusely infiltrating the dermis and cutaneous vessels. Despite medical treatment (ALL GIMEMA 7/96) the patient died of respiratory failure and pericardial effusion within four weeks. Discussion. Skin lesions in leukemia patients can be grossly classified into two types: those occurring as a consequence of direct neoplastic cells localisation at the skin level and those occurring as reactive dermatological lesions to the leukemia itself. The latter are roughly defined as leukaemids and can manifest features of various dermatologic diseases (psoriasis, lichen ruber planus, rosacea). The former are expression of direct tumour cells localisation, both in the form of skin infiltration or of vasculitis and are clinically apparent as erythematous-papulo-nodular, ulcerative or livedo reticularis-like lesions. Dermatosis can be the only apparent clinical sign of underlying leukemia, so in the differential diagnosis of skin lesions, hematologic disease must be ruled out. The only way to obtain certain diagnosis is through histologic examination of skin-biopsy specimens. Livedo reticularis-like skin manifestations are rare in acute leukemias, with only a few cases reported up to date. They are expression of extramedullary spread of the disease and in this perspective they invariably carry a very dismal prognosis with poor survival. Our case is in line with previous reports.

References

lymphoid blasts. After Campath-1H administration a complete clearance of peripheral blasts was observed. Patient 3: A 15 years-old boy diagnosed for CALLA-positive ALL achieved CR after standard induction therapy. It was then reinforced with 3 courses of intensified consolidation therapy and allogeneic-SCT from sibling donor. Two months later a grade II-III GVHD developed (skin, liver, intestine). Seven months later a relapse occurred with severe pancytopenia, splenomegaly, and 30% of marrow blasts infiltration. After administration of campath-1H, we observed a spleen size reduction (from 5 to 2 cm from the costal margin) and we documented a significant GVHD grade reduction (from grade III to grade II). No significant toxicity was observed, with the exception of infusion reaction (fever and chills) in the third patient. In our opinion Campath-1H may have clinical effects both regarding leukemic lymphoblasts clearance and GVHD reduction and larger clinical trials are warranted in order to define its role in these two subsets of patients.

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PU019
DE NOVO ACUTE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA WITH T(14;18) AND BCR/ABL REARRANGEMENT: A CASE REPORT

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The t(14;18)(q32;q21) translocation is the most common translocation in B-cell malignancies; in particular, it is found in about 80% of follicular lymphomas, being the chromosomal hallmark of this tumor, and in about 20% of diffuse large B-cell lymphomas. Only few cases of t(14;18) de novo B-acute lymphoid leukemia (B-ALL) have been described. Most of these cases presented additional chromosomal abnormalities, often involving band 8q24 and/or c-myc rearrangement and had a very aggressive clinical course. The CNS involvement seem to be a frequent event, despite of adequate prophylaxis. The association between t(14;18) and bcr/abl rearrangement has never been described. We report on a de novo B-ALL carrying both t(14;18) and bcr/abl rearrangement. The patient presented with pre-B ALL, L2 subtype. The karyotype was: t(14;18)(q32;q31). RT-PCR showed bcl2/IgH and bcr/abl rearrangements. We administered a standard induction therapy obtaining a complete remission (CR). Three intensified consolidation courses (including high dose cytarabine and high dose methotrexate) were then administered. Bone marrow harvest and autologous bone marrow transplantation were then performed, lacking a HLA-matched donor. Twelve months after the first documentation of CR, the patient relapsed. The karyotype was 46,XX, t(1,5)(p32;q31), del(12)(p11;p13) (14/15); RT-PCR showed the bcl2/IgH rearrangement. The bcr/abl fusion transcript was not detectable. Salvage therapy with daunoXome and cytarabine was then administered, obtaining a second molecular CR. Two months later, the second relapse occurred. The karyotype was: t(1,5)(p32;q31), del(12)(p11;p13)(29/30). RT-PCR showed the bcl2/IgH rearrangement. We then administered as compassionate Campath-1H, 30 mg for 5 doses, obtaining a peripheral blood blast clearance. Despite of a neuro-meningeal prophylaxis, there were signs of CNS involvement at the second relapse. The patients died 17 months after the diagnosis. This is the first case of ALL in which t(14;18) and bcr/abl rearrangement were associated. Although a sequence of molecular events cannot be hypothesised, it is possible a dominant role of bcl2/IgH, since it was present in all the disease phases.

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LYMPHOMAS

Non-Hodgkin’s Lymphomas

PU020
PRIMARY CARDIAC LYMPHOMA: A SINGLE CASE REPORT
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Primary Cardiac Lymphoma (PCL) is classically defined as an extranodal non-Hodgkin’s lymphoma exclusively located in the heart and/or pericardium. PCL is difficult to diagnose, especially during the early stage of the disease, because of its non-specific clinical manifestations, the limited possibility of using non-invasive diagnostic techniques, and difficulties or delays in applying invasive methods. The malignancy of its histotypes and its delicate location are responsible for its rapid and frequently unfavorable evolution. Ante mortem diagnosis and response to therapy have rarely been reported. We report the case of a 68 year-old male patient with non Hodgkin’s lymphoma of probable primary cardiac localization and with subsequent extension to peripheral lymph nodes. He had had unspecified cardiologic disturbances for 4 months accompanied by a febrile syndrome. The later appearance of left axillary lymph nodes led to a biopsy which allowed a diagnosis of diffuse non Hodgkin’s lymphoma with large immunoblastic cells CD20++, CD30—. A subcutaneous left atrial mass (7.3 x 6.9 cm) with invasion into the other chambers and a slight dilatation of Wirsung duct inside a dysomogeneous pancreas. The diagnosis of primary cardiac lymphoma was made following biopsy of the heart (catheterization of superior vena cava). Bone marrow biopsy was positive for LNH localization. Chemotherapy was immediately started with CHOP-like schedule. After two courses of chemotherapy, the intracardiac tumor progressed quickly (area 38 cm² e volume 144 cm³) with superior vena cava syndrome. We proceeded to a debulking treatment of heart, in emergency, with external beam radiotherapy even if we could not find, in the available literature, any patient who had ever received a heart irradiation. The correct treatment setup was checked by an x-ray film. Irradiation reduced the intracardiac tumor by 70% as demonstrated by echocardiographic controls and by spiral CT scanning of the chest. All the thoracic symptoms showed remarkable improvement. However, two months later, the disease progressed, despite chemotherapy to the pancreas and the patient died soon after a state of extreme low output. It is interesting that in this patient PCL was diagnosed ante mortem, unlike the other cases in which the disease was confirmed only after autopsy.

References
megaly. In this phase the patient was submitted only to observational follow-up. One month later the patient presented fever (38°C), pharyngodynia, left tonsil hypertrophy and submandibular lymphadenomegaly not responsive to antibiotic therapy. A fine needle aspiration biopsy and an incisional biopsy was performed on a submandibular lymph node and on tonsil respectively. Flow cytometry, the histopatological analysis and immunohistochemistry suggested a T lymphoblastic lymphoma diagnosis, CD7, CD5, CD2, cyCD3,CD4, CD8, Tdt positive, CD34+, with highly proliferating cells (Ki 67- MIB-1: 1-90%). A new total body CT control showed mediastinal, abdominal, retroperitoneal and inguinal lymphadenomegalias. Bone marrow biopsy confirmed the chronic myelogenous leukemia-like myeloproliferative disorder diagnosis. Bone marrow infiltrate by lymphomatous cells was absent. Between January and February 2003 the patient was submitted to 4 weekly chemotherapy cycles with daunorubicin, cyclophosphamide, vincristine, prednisone, and L-asparaginase for 7 days. Also intrathecal therapy with Aracytin and Prednisone was performed every two weeks. Consolidation therapy was performed by high dose Aracytin and Vepesid before and by Aracytin and Idarubicin after. The patient was in complete bone marrow and lymph node remission, at last follow-up (May 2003). The T-lymphoblastic lymphoma associated with t(8:13) and myeloproliferative disorder is a distinct clinicopathological entity. It presents at onset with myeloid cells and eosinophil hyperplasia in bone marrow and in the peripheral blood, associated with lymphadenomegaly. In our case we remark the unusual presentation with only leukocytosis in peripheral blood and myeloid hyperplasia in bone marrow one month before lymphadenomegaly onset, without bone marrow and peripheral blood eosinophilia. This confirms the pivotal role of cytogenetic and molecular biology tests in early diagnosis and in differential diagnosis of myeloproliferative and lymphoproliferative disorders, also with unusual presentation. Our experience, in accordance with the literature, confirms the efficacy of acute leukemia therapeutic strategies in this double-faceted disease, usually non responding to classical lymphoma therapies.

**PUO22**

**PRIMARY THIRD VENTRICLE NON HODGKIN LYMPHOMA WITH DIABETES INSIPIDUS: A CASE REPORT**

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Primary central nervous system lymphomas (PCNSL) are uncommon tumours of the CNS that account for less than 1% of malignant non Hodgkin lymphomas and their presentation as solitary hypothalamic-third ventricle mass can be considered exceptional. We report the case of a 53-year-old woman with progressive disorientation, mental confusion and polyuria and polydipsia. Measurements of 24 hours’ urine output and screening of urine density confirmed the diagnosis of diabetes insipidus. A computed tomography (CT) scan demonstrated a normodense, homogenously enhanced mass lesion involving the suprasellar region and extending to the third ventricle. Magnetic resonance (MR) images confirmed an isodense lesion of 3.5 cm in diameter. No ventricular dilatation was noted. Histological diagnosis proved to be a B-cell (CD20 positive) phenotype high grade diffuse non Hodgkin lymphoma. Whole body CT scan, clinical examination and bone marrow biopsy demonstrated no other site of disease. Patients was treated with high dose dexamethasone (40 mg/day for 5 days) with dramatic regression but not polyuria or polydipsia. On the 7th day after the discontinuance of steroid, a MR scan demonstrated the recurrence of the mass lesion: the patient was treated with high-dose methotrexate (1500 mg/m² at day 1, 15, 29, 43, 57 and 71) and high-dose cytarabine (3000 mg/m² at day 2, 16, 30, 44, 58 and 72). Patient received also antiCD20 immunotherapy (rituximab, 375 mg/m²) in day 17, 45 and 73. Post-chemotherapy MR images evidenced the disappearance of the lesion mass in suprasellar region without any enhancement after gadolinium administration. At the end of chemotherapy she received adjuvant whole brain radiotherapy (3000 cGy fractionated into 20 sessions plus a booster of 1000 additional cGy over the third ventricle. At present time (nine month later radiotherapy) patient is in complete remission without any neurological deficit but diabetes insipidus required hormonal replacement therapy. The review of literature confirm that PCNHL as unique lesion of third ventricle are very rare and homogeneous isodense mass with marked enhancement after gadolinium administration is the common MR data. Chemotherapy plus radiotherapy is considered the elective treatment but due to the limited clinical experience there is not a standard combination schedule.

**PUO23**

**PRIMARY CUTANEOUS B-CELL LYMPHOMA: TWO CASE REPORTS**

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Primary cutaneous B-cell lymphoma (PCBCL) are rare and constitute approximately 5-10% of all cutaneous lymphomas. In localized PCBCL radiotherapy can be
A CASE OF FOLLICULAR LYMPHOMA PRESENTING AS FIRST MANIFESTATION OF CROHN’S DISEASE

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In non-Hodgkin’s lymphomas (NHL) the gastrointestinal tract is affected in 50% of cases; the most frequent localizations are in the stomach, followed by the intestinal tract. Our case report concerns a patient affected by abdominal pain, vomiting and diarrhea. The patient was subjected to medical investigation and biopsy of a skin lesion that led to the diagnosis of follicular lymphoma. The patient was subsequently diagnosed with Crohn’s disease. The treatment consisted of chemotherapy plus rituximab. In conclusion, we report this case in order to describe an unusual localization, only cutaneous large B-cell lymphomas CD30+, that had a good response to chemotherapy.

PU025
Efficacy and Toxicity of Modified-FND (M-FND) vs CHOP Regimen in Patients with Previously Untreated Follicular Non-Hodgkin’s Lymphoma


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Background: follicular non-Hodgkin’s lymphomas (FL) are initially responsive to broad range of chemothera-

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Fludarabine phosphate is a purine analogue commonly used in the treatment of low-grade lymphoid malignancies. Myelosuppression is the main toxicity, although increased incidence of autoimmune hemolytic anemia (AHE) is frequently reported. CLL by itself is associated with a high risk of autoimmune disorders, mainly AHE. However, immune thrombocytopenia also, has been reported to occur in CLL patients treated with fludarabine. Here we report a further case of this complication in a patient without previous autoimmune disorders. The patient, a 57 year old man with indolent B-CLL developed, after a 2 year follow up without therapy, a more aggressive disease with diffuse nodes, high WBC count (100 x 10^9/L), anemia (Hb 10 g/dL), hypogammaglobulinemia but normal platelet count (150 x 10^9/L). He was treated with Fludarabine-cyclophosphamide for three days: on day 4 he developed severe thrombocytopenia (7 x 10^9/L) with mucosal bleeding. Anemia worsening was also evident at day 14 and transfusion support with both platelets and packed red cells was required. Direct antiglobulin test on erythrocytes was still negative but anti-platelet auto-antibodies were detected. A complete platelet recovery was achieved after high dose steroid + Ig therapy and a month later anti platelet autoantibodies were no longer detectable. The patient was treated with 6 CVP (Cyt,Vcr,Pdn) courses and achieved a CR, persisting at 12+ months.

The pathogenesis of autoimmune thrombocytopenia, like that of AHE is still unknown, however a role of T-cell depletion is suspected. Indeed, fludarabine induced reduction of CD4+ T cell (down to 10% in FL, 20% in FLN) have been reported to occur in FLN patients with AIDS and the frequent hypogammaglobulinemia due to the primary disease may play a role in the rising of unsuppressed lymphocyte clones. However, the very rapid occurrence of thrombocytopenia (4 days after the start of fludarabine) and the fast disappearance of anti-platelet antibodies 1 month after therapy discontinuation rise the possibility of a direct role of fludarabine by aptene-induced immune thrombocytopenia as rarely observed with non-cytotoxic drugs. In this case, thrombocytopenia should immediately recur after a new drug exposition and this would be a more definite controindication to further fludarabine therapy.
Background. In the latest years Rituximab has been widely tested as single agent or in combination with the CHOP regimen for the treatment of patients with newly diagnosed aggressive NHL such as diffuse large B-cell lymphoma (DLBCL) or mantle cell lymphoma. Synergism may be demonstrated with other chemotherapy regimens. In this phase 2 study eleven patients with previously untreated DLBCL received Rituximab from the 9th to 12th weeks of the MACOP-B regimen as intensification. Objectives. To evaluate the safety and efficacy of the addition of Rituximab to the conventional MACOP-B regimen for young adult patients with previously untreated DLBCL. Design and Methods. From February 2002 to February 2003 eleven consecutive patients (male/female: 4/7) with a median age of 42 years (range 32-57 years) and newly diagnosed CD20+ patients were treated with Rituximab at the dose of 375 mg/sm weekly for 4 weeks in conjunction with the MACOP-B regimen, from the 9th to 12th weeks of therapy. Response was evaluated before the 1st administration of Rituximab and at the end of the combination regimen. Additive radiotherapy was planned for 6 patients after the immuno-chemotherapy programme. The patient characteristics at diagnosis were as follows: Ann-Arbor stage (II: n=2; III: n=2; IV: n=6), B symptoms (n=3), bone marrow involvement (n=4), lymph nodes involved > 2 sites (n=7), extranodal involvement > 1 site (n=6), bulky disease (n=5), IPI score: 0: n=1; 1: n=3; 2: n=6; 3: n=1. Results. Of the 11 pts, 8 were in partial remission at the beginning of the Rituximab and 3 were in complete remission. Of the 9 pts evaluable at the TC evaluation performed 1 month after the MACOP-B/Rituximab, 8 were in complete remission and 1 patient showed progressive disease. Two patients died during treatment with Rituximab: 1 of acute liver failure following HCV-related hepatitis and 1 of acute heath failure 3 days after the end of the immuno-chemotherapy programme (this patient had been previously treated with local radiotherapy for a rhinopharynx solid tumor). As of May 2003 all 8 responders are in complete remission with a median follow-up of 7 months (range 3-15 months). Conclusions. Although a significantly larger number of patients and longer follow-up are requested, the MACOP-B/Rituximab schedule seems to be an effective and feasible combination for the treatment of aggressive B lymphomas.
PU029
HEPATITIS C VIRUS AND NON HODGKIN’S LYMPHOMA
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Whether hepatitis C virus (HCV) may have an etiologic role in non Hodgkin’s lymphoma (NHL) still remains matter of debate. Prevalence of HCV infection in NHL was reported in Italy, Japan and USA, non in other countries. Controversies data exist on the histology of HCV-related NHL, low or high grade at onset as well as on nodal or extra nodal localization. We studied 109 consecutive patients with NHL with the aim of clarifying prevalence of HCV infection, histotype, site of the primary localization, HCV genotype and host genetic factors, HLA class I and II, associated with the development of HCV-related NHL. HCV infection was detected in 19/109 (17%) versus a prevalence < 9% in our area. Sixty of 109 had low grade and 49/109 high grade histology. Eleven of the former group (18%) and 8 of the latter (16%) were HCV infected. Extranodal NHL were significantly higher in HCV infected patients, 5/14 (36%) independently of low and high grade histology. Concerning the HCV genotype, type 1b was prevalent. Any aplotype confer susceptibility to HCV related NHL. In conclusion, our findings support an increased prevalence of HCV in patients with NHL. No difference emerged according to histotype, whereas extranodal involvement appeared frequent primary localization in HCV-related NHL.

PU030
SEVERE AND PROLONGED HEMATOLOGICAL TOXICITY AFTER IMMUNO-CHEMOTHERAPY WITH RITUXIMAB AND FLUDARABINE; A REPORT OF TWO CASES
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In literature there are recent studies that show the efficacy of combination therapy with Rituximab and Fludarabine in low grade non-Hodgkin lymphoma. Nevertheless such therapy presents a stronger hematologic toxicity respect chemotherapy or immunotherapy alone. According to NCI-CTC criteria the immunochemotherapy presents an hematologic toxicity in the following percentage: grade 1-2 neutropenia in 20% of the cases, grade 3-4 in 50% of the cases; grade 1-2 thrombocytopenia in 20% of the cases and grade 3-4 in 5% of the patients. We describe two cases of prolonged severe pancytopenia observed for several months after the stop of the immunochemotherapy. The first patient was a 74 years old woman with an extranodal low grade non Hodgkin B-lymphoma started in 1999 with cutaneous and bone marrow localizations, initially treated with clobucabril at standard dose with complete remission. At the relapse in May 2002 the patient, with skin and nodes involvement, was treated with Rituximab at the dose of 375 mg/m² (day 1) and Fludarabine 25 mg/m² i.v. (day 2-6). After three course of therapy the patient showed a severe hematologicat toxicity with grade 4 neutropenia and thrombocytopenia and grade 2 anemia. A therapy support was needed with red blood cell and platelet transfusion and with daily administration of G-CSF and EPO. After 6 months the patient is free from transfusion but still presents grade 1 thrombocytopenia and needs bone marrow stimulation with G-CSF and EPO every other day to maintain adequate values of white blood cells and hemoglobin. The second case regards a 62 years old woman affected by a B non-Hodgkin low grade lymphoma involving spleen and bone marrow. The onset of the disease was in August 2000 with massive splenomegaly and leucocytosis; the patient was treated with three course of Fludarabine as a single agent without a significative toxicity. The fourth course was preceded by a standard dose of Rituximab; after two weeks the patient developed a severe pancytopenia treated with growth factors and platelets transfusions. This hematologic picture went on over our expectations turning in to normality after six months. Both the patients reached a complete remission of disease which is still present. On our opinion the interest of these cases is the prolonged severe cytoppenia observed for several months; its explanation is not clear.

PU031
NON HODGKIN LYMPHOMA COEXISTING WITH CHRONIC MYELOMONOCYTIC LEUKEMIA; REPORT OF A CASE
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F.V., male, aged 77, came to our observation in march 2002 for thrombocytopenia and mild leucocytosis. Peripheral blood counts were: wbc: 14000/mmc (30% neutrophils, 1% basophils, 30% lymphocytes, 49% monocytes and myelomonocytes), Hb. 10.8 g/dL, Plt: 14000/m3. Phisy-call examination resulted in liver and spleen enlargement, lymph nodes palpable in all superficial sites. TC scan was performed and showed lymph node involvement at cervical, axillar, mediastinical and abdominal sites, liver and spleen enlargement. Bone marrow aspiration results in high cellularity, monocytosis from promonoblast to monocyte accounting for 31% of the total, displastic features of the erythroid clone, lymphocyte population in normal
nation has been demonstrated in numerous animal
cancer has achieved very limited success to date. Yet, can-
nonion in history. Instead, active immunotherapy of can-
as been one of the most successful medical interven-

Liso A,* Benedetti R,* Castiglione F,° Toschi F,°
QUANTITATIVE MODEL
VACCINATION AGAINST LYMPHOMA: NEW INSIGHTS FROM A

monocytic leukemia.

B-lineage coexisting at diagnosis with chronic myelo-
edgment, the first report of a high grade lymphoma of the
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phoid and myeloid disease was found. All lymphomas were
serie of 1198 patients affected by mds (Forlensa, Leukemia
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is rare. Only three are the published cases, regardind in two
of them lymphomas of T-lineage, and the third a breast B-
lymphoma. Coexisting untreated lymphoproliferative dis-
ease and myelodisplasia has been reported as casual in a
series of 1198 patients affected by mds (Forlensa, Leukemia
and Lymphoma 1996). Only in 5 CM ML concomitant lymph-
phoid and myeloid disease was found. All lymphomas were
of the B-lineage and were low grade. This is, to our knowl-
edgment, the first report of a high grade lymphoma of the
B-lineage coexisting at diagnosis with chronic myelo-
monocytic leukemia.

PU032
VACCINATION AGAINST LYMPHOMA: NEW INSIGHTS FROM A
QUANTITATIVE MODEL
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Prophylactic vaccination against infectious disease has been one of the most sucessful medical interven-
tion in history. Instead, active immunotherapy of can-
cer has achieved very limited success to date. Yet, can-
cer eradication using tumor-associated antigen vacci-
nation has been demonstrated in numerous animal
models. In vitro systems and human clinical trials indi-
cate that T cells recognize antigenic fragments derived
from gene products expressed by tumors. Nevertheless,
results from clinical trials have been largely disap-
pointing, since vaccine protocols designed to elicit anti-
tumor immune responses have, in the majority of cas-
es, failed in producing tumor eradication and/or in pro-
longing patient survival. Therefore, to explain how a
remarkably complex system recognize and protects
against infectious agents and generally fail to respond
to anti-cancer vaccines, we used a computational mod-
el. The model entails the major immune cell types and
it is equipped with immunological processes as hema-
topoesis, thymus selection, hypermutation of antibod-
ies and antigen processing-presentation to killer T cells.
We show that competition rather than help develops
over traditional vaccination protocols with tumor asso-
ciated antigens. We also investigated our hypothesis in
DNA vaccination protocols against murine lymphoma
38C13. The results of our study have direct implication
in the design of new anti-cancer vaccines.

PU033
ACOP-B REGIME FOLLOWED BY INVOLVED FIELD RADIATION
THERAPY IN LIMITED STAGE AGGRESSIVE NON-HODGKIN’S
LYMPHOMA. A SINGLE CENTER RETROSPECTIVE STUDY
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Background: This study relates our experience in the
treatment of limited-stage diffuse large cell lymphoma.
Patients and methods: Seventyseven consecutive patients with a median age of 50 years (range 18-85)
with limited stage diffuse large cell lymphoma diag-
nosed and treated at a single institution between 1993
and 2002, were reviewed. Only patients with nodal or
extranodal stage I A or II A were included. All patients
received first line therapy with ACOP-B regimen
(adryamicin 50 mg/m² day 1, 15, 29, cyclophosphamide
350 mg/m² day 1, 15, 29, vincristine 1.4 mg/m² day 8,
22, 36, bleomicine 10 mg/m² day 8, 22, 36, predinisone
50 mg/day day 1 to 42), followed by involved field
radiotherapy (36 Gy). Results: The treatment was well
 tolerated, with minimal hematologic toxicity (grade 1-
2) in 20% of patients, extrahematologic consisted of
alopecia and grade 3 mucositis after radiotherapy of
Waldeyer’s ring in 80% of patients. Complete remis-
son was achieved in the 95% after the combined treat-
ment, no difference between I or II stage was observed.
After a median follow-up of 4.2 years (range 0.1-9.5),
Kaplan-Meier probability of overall and event free sur-
vival were 85% and 80% respectively. In this group
there were 11 deaths, 3 in progression disease, and 8
due to disease unrelated causes. Conclusions: These

haematologica vol. 88[suppl. n. 15]:october 2003
Introduction: Primitive bone NHL constitutes about 5% of extranodal lymphomas. It is difficult to decide the optimal treatment for this type of lymphoma because of its rarity. Patients and methods: Between March 1993 and May 2003, eighteen previously untreated patients (12 male and 6 female; median age 41 years, range 18-78) with primary NHL of bone were followed at our institution. Sixteen patients had diffuse large cell lymphoma, two low grade istotype. The diagnosis was obtained with open curretting biopsies in all patients. Bone involvement was multifocal in two patients and solitary in the others, involving scapula (n=5), femur (n=5), humerus (n=3), vertebral bones (n=2), tibia (n=1), radio (n=1), clavicula (n=1). The affected bone exhibited a lytic appearance on plain film and CT scans. Bone marrow biopsy was positive in one patient. Eleven patients had stage I disease and six stage II. Bulky disease occurred in ten patients (55%), LDH was elevated in eight patients (44%), IPI was intermediate-high in seven patients and low-intermediate in other seven ones. Chemotherapy, according to disease stage and patient age, were ACOP-B in six cases, VACOP-B in four cases, MiniCEOP in two cases, other schedule containing antracycline in four cases and High Dose Sequential chemotherapy followed by autologous PBSC transplantation in other two patients. Involved field radiotherapy (36-46) was administered to fifteen patients (83%). Results: The treatment has been well tolerated, seventeen patients (94%) achieved a complete remission and the other one a partial response. After treatment, three patients relapsed, two in the CNS and died for progression disease, one patient developed colon cancer 2 years after radiotherapy and died. After a median follow-up of 5.5 years (range 0,5-10), 14/18 patients are alive (77%). Conclusions: these results underline that multilagent chemotherapy combined with involved field radiotherapy improves prognosis and course of this disease, considered a systemic disease.

Monoclonal antibody therapy with anti-CD52 (Campath 1H, Alemtuzumab) is now a used and promising approach to the treatment of some non-hodgkin lymphoma. Nevertheless is well known its immunospressive effect with an important reduction of CD4 T lymphocyte and an increased risk of fungal, bacterial and viral infection. In the literature are also described some cases of secondary neoplasia like lymphoma but there are no segnalation of Kaposi’s sarcoma developed after this kind of therapy. We describe the case of a 47 years old man affected by a small and large cell diffuse non Hodgkin lymphoma CD20+ in stage IV for bone marrow infiltration treated, after the onset in February 2002, with 6 course of rituximab plus CHOP. After this therapy for the persistence of minimal bone marrow disease the patient was treated with HD-ARA-C plus rituximab at day 1 and 9 in order to perform stem cell collection. In September 2002 was conducted the stem cell transplantation after BEAM conditioning regimen without any important toxicity. In December 2002 the revaluation showed a residual bone marrow disease quantified in about 20% of monoclonal B-lymphocyte. An additional therapy with campath 1H was started in January 2003 in order to eliminate the residual disease. After three weeks of subcutaneous therapy at standard dose the treatment was stopped for grade 4 hematological toxicity that needed transfusion therapy and stimulation with G-CSF; the CD4 count at this time was 2 cells/microlitre. Nevertheless the resolution of the neutropenia the patient was admitted to the hospital for the presence of respiratory symptoms and fever. Many severe infective complications occurred during the hospitalization: a pulmonary aspergillosis, an enterobacter and pseudomonas sepsis and multiple cerebral lesions with the radiological aspect of toxoplasma localizations. These infective complications caused a worsening of the hematological conditions with a severe pancytopenia that went on for several weeks in spite of stimulation with growth factors and two successive infusions of autologous stem cells. During this period appeared on the leg some purple skin nodes; a skin biopsy led to the diagnosis of Kaposi’s angiomatoid sarcoma in nodular phase. An antiretroval therapy was started considering the possibility of viral origin of this kind of tumor; it was no possibi to associate any kind of antiblastic drugs for the
Vanishing bile duct syndrome (VBD) is a dramatic event in hepatic disorders, often resulting in fatal outcome. VBD has been described in association with Hodgkin disease, particularly at diagnosis, but the pathogenetic correlation between the two clinical entities is unknown. We were not aware of cases of VBD occurring in patients with other hematological neoplasms. We describe a case of anaplastic T cell lymphoma presenting with VBD syndrome. A 28-years old man was admitted to our Institution on April 2003 with hepatosplenomegaly, lymphadenopaties (lateral-cervical, retroperitoneal), leucocytosis (63.48 × 10^9/L) and of platelets (PLT > 20 × 10^9) required 9 and 12 d post-transplant, respectively. To prevent HBV reactivation, the patient received oral lamivudine, that, will continue 18 months at least. The procedure was altogether well tolerated. This therapeutic approach is already known in the treatment of non Hodgkin lymphoma, but at present, there isn’t evidences of literature for PHL. The patient undergone follow-up for 6 months since autologous transplantation and nowadays he’s in complete remission of disease.

PU037
VANISHING BILE DUCT SYNDROME IN AN ANAPLASTIC T-CELL LYMPHOMA PATIENT WITH ABNORMAL KARYOTYPE: FIRST CASE REPORT


Vanishing bile duct (VBD) syndrome is a dramatic event in hepatic disorders, often resulting in fatal outcome. VBD has been described in association with Hodgkin disease, particularly at diagnosis, but the pathogenetic correlation between the two clinical entities is unknown. We were not aware of cases of VBD occurring in patients with other hematological neoplasms. We describe a case of anaplastic T cell lymphoma presenting with VBD syndrome. A 28-years old man was admitted to our Institution on April 2003 with hepatosplenomegaly, lymphadenopaties (lateral-cervical, retroperitoneal), leucocytosis (63.48 × 10^9/L) and of platelets (PLT > 20 × 10^9) required 9 and 12 d post-transplant, respectively. To prevent HBV reactivation, the patient received oral lamivudine, that, will continue 18 months at least. The procedure was altogether well tolerated. This therapeutic approach is already known in the treatment of non Hodgkin lymphoma, but at present, there isn’t evidences of literature for PHL. The patient undergone follow-up for 6 months since autologous transplantation and nowadays he’s in complete remission of disease.

PU036
A CASE OF PRIMARY NON HODGKIN LYMPHOMA OF THE LIVER IN HBSAG POSITIVE PATIENT, TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

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Primary hepatic non-Hodgkin lymphoma (PHL) is a rare lymphoproliferative disorder. Diagnosis is difficult and histological examination is essential to confirm it. Diffuse large cell lymphoma is the most common histologic subtype. Several cases of PHL develop in patients with chronic hepatitis C virus infection; relationship with lymphoma is unclear but the frequent association suggests that the virus may play some roles in the pathogenesis of PHL. Rare cases of PHL associated with hepatitis B virus infection have been described. Surgery, chemotherapy and radiotherapy have been used alone or in combination as treatment but the outcome is generally poor, with a median survival of 3.7 months. Recent reports suggest that combination chemotherapy (anthracycline-based regimens) is the most appropriate treatment, with or without surgery and/or radiotherapy, with a CR rate of 83.3%, a 5-year OS of 83.1%, a 5-year recurrence free survival rate of 83.1% and a 5-year failure free survival rate of 70.1%. We describe a case of 65-year-old male, with negative remote pathologic anamnesis, suffering from abdominal pain and jaundice. Physical examination revealed hepatomegaly and laboratory findings showed coelastic markers elevation. Furthermore, the patient was HBsAg positive. Radiological findings showed a marked dilatation of intrahepatic bile ducts and a single liver lesion with maximal extension of 9 cm, suggesting for cholangiocarcinoma. Histological examination only revealed diffuse B large cell non-Hodgkin lymphoma (WHO, Kiel) WF: G. So diagnosis was primary non Hodgkin lymphoma of the liver, since the lesion was the only one present. Patient, according to literature, received cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP). After six cycles of chemotherapy the computed tomodography showed a partial remission of disease, so an autologous transplant was planned. Patient received mobilization chemotherapy with high dose cyclophosphamide and subsequent peripheral blood stem cell transplantation. After preparative regimen with CCNU, VP-16, ARA-C and melphalan (BEAM) the patient received 4.5 × 10^9 CD 34+/kg body weight. Recovery of neutrophils (PMN > 0.5 × 10^9/L) and of platelets (PLT > 20 × 10^9) required 9 and 12 d post-transplant, respectively. To prevent HBV reactivation, the patient received oral lamivudine, that, will continue 18 months at least. The procedure was altogether well tolerated. This therapeutic approach is already known in the treatment of non Hodgkin lymphoma, but at present, there isn’t evidences of literature for PHL. The patient undergone follow-up for 6 months since autologous transplantation and nowadays he’s in complete remission of disease.
patients. Oral FLU is administered at 40 mg/m²/d CY in pretreated indolent non-Hodgkin’s lymphoma to evaluate the efficacy and safety of the combination of oral FLU and oral CY at 300 mg/m²/d, days 1 through 3, repeated every 28 days for 6 courses. Efficacy endpoints include overall response rate, minimal residual disease assessment, duration of response, time to treatment failure and treatment free interval. Response evaluation is performed 2 months after the end of treatment and confirmed 3 months after. Patients’ characteristics included median age of 61.8 years (54-73); 7 male, 3 female; 8 pts were in stage IV and 2 in stage II. According to histology there were 9 follicular lymphoma, and 1 immunocytoma. The patients underwent to a mean of 2.5 previous chemotherapy regimens (range, 2-4). So far 10 patients were enrolled. Response was assessed on 8 patients: 5 CR (62.5%), 2 SD (25%) and 1 PD(12.5%). Two patients died during follow up (one of progression disease at months 4, one of cardiovascular accident at month 7). Safety data were evaluated on every cycle in all patients. The toxicity was mainly hematological: NCI grade 3/4 neutropenia (20.8% of the cycles), anemia (8.3%), and thrombocytopenia (8.3%), PCP prophylaxis were recommended. Infective episodes were not registered. Gastrointestinal toxicity was mild and easily controlled by common antiemetic drugs. In conclusion, this analysis suggests that this oral combination treatment is a promising second-line therapy in indolent NHL, with 62.5% overall response rate.

PU039
MAGNETIC-RESONANCE-IMAGING ON PRIMARY BREAST LYMPHOMAS: MANAGEMENT OF 6 CASES DIAGNOSED AND TREATED AT THE EUROPEAN INSTITUTE OF ONCOLOGY
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Introduction: Primary lymphoma of the breast (PBL) is a rare clinic-pathological entity that accounts for 0.04–0.5% of all breast malignancies and less than 1% of all non-Hodgkin’s lymphomas. The mammographic and ultrasonographic (US) features of breast lymphoma are non-specific. Gadolinium enhanced magnetic resonance imaging (MRI) of the breast has been used in patients with PBL to identify small multifocal foci in dense breast. Here we report our experience with MRI in the diagnosis and follow-up of PBL. Patients and Methods: From June 2001 to December 2002, 6 cases of primary non-Hodgkin’s lymphoma of the breast were evaluated using MR-imaging of both breasts after diagnosis, after 2 cycles of chemotherapy, 40 days after the end of radiotherapy and at follow-up. Images were acquired on a T1-weighted fat-suppressed FSPGR 3D sequence. The slice thickness was chosen between 1.5 and 3 mm depending on breast size. The sequence was performed once before and five times after intravenous contrast (Gd-DTPA 0.2 mmol/kg) injection. All patients were female, their median age was 58 years (range 34-65); 4 patients were in stage I (Ann Arbor Stage) and 2 in stage IIE. Histological diagnosis, according to the WHO classification, was diffuse large B-cell in all patients. All patients received ACOD (CHOP-like chemotherapy) for 4-6 cycles followed by 44-46 Gy radiotherapy of the breast and involved nodes if indicated. Results: In 5/6 cases mammography, US and MRI identified the lesion, always without microcalcification and without desmoplastic reaction. Only in one case mammography showed asimmetric distribution of glandular tissue in the breast, without a detectable
nodule; in this case US and MRI identified the nodular lesion. In one patient MRI demonstrated the multifocality of the pathology, identifying several micronodules disseminated in the whole breast (not seen at conventional imaging) e distinguishing between nodular disease and fibroadenoma, as showed at US. In another case MRI demonstrated the presence, after nodulectomy, of a round shaped enhancement (1 cm) due to residual disease, that mammography and US diagnosed as fibrotic tissue. At last follow-up all patients achieved complete clinical remission, without radiological evidence of disease at MRI. Conclusion: MR-imaging is more accurate than mammography and US at defining the true extent of the disease, even after surgery, and seems to highlight multifocal disease better. In primary breast lymphoma, MRI should be recommended as the radiological procedure of choice for defining the local extent and monitoring response to the treatment of this uncommon disease.

PU040
PRIMARY CUTANEOUS NATURAL KILLER CELL LYMPHOMAS:
A DESCRIPTION OF TWO CASES
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Introduction. CD 56+ lymphomas derived from natural killer (NK) cell lineage are rarely encountered in Western populations, affecting elderly patients in Asia and South America. The majority of reported cases are lymphomas of the nasal cavity; however, various organs can be involved (skin, mucous membranes, lymph nodes, bone marrow) and a leukemic evolution can be observed. Prognosis is unfavorable and standard chemotherapy regimens cure these lymphomas very rarely. Case reports. We describe two cases of primary cutaneous NK-lymphomas in elderly Caucasian males. Skin lesions appeared as red-brown patches and/or plaques and nodules located above all on face and trunk. Cutaneous biopsies showed an infiltrate characterized by atypical medium-sized cells in the dermis, especially around small vessels. These cells were sCD3/CD3e and CD56+ in the first case and sCD3+ and CD56+ in the second case; moreover, in both cases these cells were CD30 negative and presented an high mitotic index (Ki-67 >30%). Gene rearrangement for TCR was negative in the first case and positive in the second case; in situ hybridization showed no EBV-related genes in both of them. A diagnosis of blastic NK-lymphoma and T/NK-lymphoma was made in the first and second case, respectively. In both cases, despite the treatment, the disease progressed from skin to rhinopharynx, lymph nodes and bone marrow involvement, with the death supervening in a leukemic phase a few months from diagnosis. Conclusions. Cutaneous involvement in NK-lymphomas is important as skin lesions are very often the first appearance of the disease and they are easily accessible for a diagnostic biopitic specimen. Prognosis is very poor with standard chemotherapy and aggressive schedules of treatment should be considered in the future.

PU041
SALVAGE AND MOBILIZING REGIMEN FOLLOWED BY HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANT FOR RELAPSED HIGH GRADE NON-HODGKIN’S LYMPHOMA
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Objectives: High dose chemotherapy with autologous hematopoietic stem cell transplantation (HSCT) is an effective treatment for patients with relapsed high grade non-Hodgkin’s lymphoma. We evaluated the role of the IEV regimen as salvage and mobilization treatment, followed by high dose therapy and HSCT, in 16 consecutive patients. Patients and Methods: A three drug combination of ifosfamide 2500 mg/m^2 on day 1-3, Etoposide 150 mg/m^2 on day 1-3, Epirubicine 100 mg/m^2 on day 1 (IEV) was used to treat 16 patients with high-grade non Hodgkin’s lymphoma; 14 relapsed (median CR duration after first line therapy 14.8 mos) and 2 primary refractory ones. The pts received 3 monthly IEV courses. The second course was followed by G-CSF administration and CD34+ cells collection. Autologous HSCT, following BEAC conditioning (BCNU 200mg/m^2 on day 2, Etoposide 300 mg/m^2 on day 1-4, Ara-C 300 mg/m^2 on day 1-4, Cyclophosphamide 45 mg/kg on day 1-4), was performed in all the 16 pts, within 2 months after the last IEV course. Results: 5/14 patients with relapsed lymphoma (36%) achieved a complete response (CR) and 9 (64%) a partial response (PR) at the end of the three IEV cycles. Of the 2 refractory pts, 1 achieved a partial response. Clinical and hematological toxicity were moderate. All the 16 patients mobilized peripheral blood progenitor cells: the median yield was 4.5x10^6 CD 34+ cells/kg (range 2.0-8.0). The 16 patients had sustained engraftment before BEAC conditioning and autologous HSCT. The median time to granulocyte recovery (0.5x10^9/L) was 10 days (range 7-15); median time to platelet recovery (20x10^9/L) was 12 days (range 9-14). Oral toxicity greater then grade I occurred in 6/16 patients (37%); no patient developed a severe hepatic toxicity, but 2
patients, with a normal baseline resting left ventricular ejection fraction (LVEF greater than 50%) before IEV and HSCT, after the treatment developed a dilated cardiomyopathy with symptoms of congestive heart failure. Complete response rate was 50% (1 refractory patient achieved a CR) and partial response rate 37% (overall response rate 87%). At present 9/16 patients are alive (56%), and 6/16 (37%) in complete response, with a median follow-up of 19 months from HSCT (range 4-58).

Conclusions: These results confirm the efficacy of the IEV regimen in the treatment of relapsed high-grade non-Hodgkin’s lymphomas, with a good response rate and mobilizing effect. The combination with high dose chemotherapy and autologous HSCT seems to be effective for these patients with a poor prognosis, and rather safe, even if the cardiotoxic risk must be carefully evaluated.

PU042

DISTRIBUTION OF HEPATITIS C VIRUS GENOTYPES IN LYMPHOPROLIFERATIVE DISORDERS

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HCV is both hepatotropic and lymphotropic and the ability of the virus to infect and to multiply in mononuclear cells, both in peripheral blood and bone marrow, has been documented. (Zignego, J Hepatol 1992) The virus has been shown to be the main etiologic agent of type II mixed cryoglobulinemia and several recent reports suggest also a pathogenic role in other lymphoproliferative diseases (LPD). (Silvestri, Blood 1996 and Mele,Blood prepublished online April 24, 2003) Epidemiological studies showing a HCV prevalence rate in LPD higher than in the general population deal with Southern Europe populations. There are six major genotypes of HCV, classified with numerals 1 to 6 (Simmonds, Hepatology 1994) and a considerable genetic variability has been shown. The distribution of HCV genotypes/subtypes shows a marked geographic variability. Type 1a is more frequent in USA; type 1a, 2a and 2b are prevalent in Japan and Taiwan; type 3 is the most common in Asia and type 4 in the Middle East. In Italy the most prevalent HCV genotype is type 1b, accounting for up to 70% of infections. In a group of 40 HCV positive LPD patients we have investigated the distribution of HCV viral genotypes and studied the correlation between the genotype and LPD histotype. Results are summarized in Table 1. The crude HCV prevalence rate in 300 LPD patients was 13.3% (40/300), and 71.4% of them were also HCV-RNA positive. Comparing the HCV prevalence rate found in LPD to that estimated for healthy people in Southern Italy (about 10%), we found it higher in B-NHL, MM, and MGUS. In patients with the Large B-cell non Hodgkin Lymphoma HCV genotype 1b was found in 11/17 (64.7%) and type 2a/2c in 5/17 (29%). In our study, the distribution of HCV genotypes in patients with LPD was similar to that known to be characteristic for our geographic area, with a clear prevalence of type 1b. Conversely, the prevalence of HCV genotype 2a/2c was unexpectedly higher than expected and almost restricted to diffuse large B-cell lymphomas. Our observations is consistent with the hypothesis that the different HCV genotypes could have different lymphotropism. Further studies are needed to clarify the role that HCV infection plays in the multistep process leading to lymphoproliferative B-cell malignancies.

Table 1.

| LPDHCV+ (%) | RNA+ (%) | HCV Genotype no analyzed
|------------|---------|----------------------
| B-NHL      | 13/17(76%) | 9/11 (81.8%) |
| MM         | 6/30(20%)  | 4/6 (66%)        |
| B-CLL      | 4/52(7.6%) | 2/4 (50%)       |
| MGUS       | 2/10(20%)  | 1 (50%)        |
| HD         | 1/48(0.2%) | 1 (0.2%)       |
| T-NHL      | 1/21(4.7%) | 1 (4.7%)      |
| Total      | 40/300(13.3%) | 23/35 (71.4%) |

References

PU043
ASSOCIATION OF RITUXIMAB WITH CHEMOTHERAPY DOES NOT INCREASE INFECTIONS. A RETROSPECTIVE ANALYSIS IN B-CELL LYMPHOMAS FROM A SINGLE CENTER
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Rituximab (R) is a new standard for B-cell lymphomas expressing CD20 antigen. Its effectiveness has been already shown both in low- and high-grade lymphomas. Besides allergic reactions and tumor lysis syndrome, infectious diseases are a major concern of this immunotherapy. We retrospectively evaluated infectious toxicity in a series followed at the IRC of Candiolo since 1999. Patients and methods. Among 153 patients affected by B cells lymphomas treated at our Institute between April 1999 and August 2002 we analyzed 138 patients homogeneously treated: 28 follicular lymphomas treated with fludarabin, mitoxantron, dexamethasone (FND+R); 88 large cell lymphomas treated with CHOP or CHOP like chemotherapy+R; 22 large cell lymphomas treated with intensified chemotherapy (HDS)+R. Mean age at diagnosis was 58. We collected data about identified infectious diseases or fever classified as fever of unknown origin (FUO). The statistical differences among homogeneous groups were determined by using the chi-square test. Results. In 41 patients treated with 4 or more cycles of CHOP chemotherapy we observed 21 infectious events (7 viral, 15 bacterial and 3 mycosis). In 47 patients treated with R-CHOP we observed 19 infectious events (7 viral, 9 bacterial and 5 mycosis). The difference was statistically significant only for bacterial infections (p <0.019). In 11 patients treated with FND chemotherapy we observed 3 bacterial events. In 17 patients treated with R-FND we observed 10 infectious events (3 viral, 6 bacterial and 1 mycosis). The difference was not statistically significant (p=0.27). In 13 patients treated with HDS we observed 7 infectious events (3 viral and 4 bacterial). In 9 patients treated with R-HDS chemotherapy we observed 4 infectious events (2 viral and 2 bacterial). The difference was not statistically significant (p = 0.67). However in this subset the R-HDS group was more frequently affected (5 vs. 0) by FUO with a statistically significant difference (p = 0.02). This difference was not clinically significant. Conclusions. This retrospective series confirms the results of the French study by Coiffier which did not show an increased toxicity in the R-group. This holds true also if we consider any of the causative agents (viral, bacterial or fungal) or the sum of the days of hospitalization due to infections. We conclude that infectious toxicity should not be overemphasized even in a population-based set.

Patients affected by aggressive non-Hodgkin's lymphoma (NHL) who have relapsed after autologous peripheral stem cell transplantation have a very poor prognosis. PEGylated liposomal doxorubicin (PLD) has been shown to have at least similar efficacy as conventional doxorubicin also in aggressive NHL even as first line therapy as well as salvage therapy, while reducing cardiac toxicity. Rituximab (RTX) is effective as single agent in relapsed or refractory HG-NHL and combination therapy with GM-CSF seems to improve the response rate. The aim of our study was to evaluate the efficacy and the tolerability of the association RTX, PLD, cyclophosphamide (CTX) and GM-CSF in patients with aggressive lymphoma as salvage therapy (post autotransplantation or in patients poor mobilizer). Fifteen patients entered the study, 13 affected by high grade NHL and 2 affected by mantle cell lymphoma with a median age of 60 years (range 28-76). Twelve out of 15 (80%) had an intermediate risk lymphoma (International prognostic index) while 3 out of 15 had a low risk lymphoma. Eleven out of 15 (73%) had received a previous treatment with autologous stem cell transplantation while only 1 received a previous allogeneic marrow transplantation. Fourteen out of 15 patients are evaluable for the response. The overall response (OR) and complete response (CR) rates were respectively, 57.1% and 42.8% respectively. With a median follow-up of 6 months (range 0.5-34) 42.8% of patients are in continuous complete remission. The main toxicity was represented by myelosuppression; grade III-IV anemia was observed in 11.1%, grade III-IV thrombocytopenia in 26.6% and grade III-IV neutropenia in 28.9%. Clinically relevant infections were recorded in only 6.6% of patients while no cardio-vascular acute adverse effects were observed; other extrahematological toxicity was negligible and consisted in diffuse dysesthesias and itch in one patient. In conclusion the association of PLD, RTX, CTX and GM-CSF seems to be effective and without relevant toxicity in a subset of patients affected by aggressive NHL with a particularly bad prognosis.
PU045
CIS-PLATINUM, IDAURICIN, PREDNISONE (CIP) AFTER THE P-VABEC REGIMEN CAN IMPROVE SURVIVAL IN ELDERLY PATIENTS WITH DIFFUSE LARGE CELL LYMPHOMA. FINAL RESULTS OF AN ITALIAN MULTICENTER RANDOMIZED STUDY
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Results: The P-VABEC regimen was found to be a safe and active chemotherapy for elderly patients (pts) with diffuse large cell lymphomas (JCO 11- 2363, 1993). However, in spite of a high rate of complete response (CR) the progression-free survival (PFS) rapidly decreased for a high incidence of progressive disease or early relapses. A phase II pilot study (Leuk Lymphoma 24-335, 1997) demonstrated that CIP regimen was an active and well tolerated therapy in pts with progressive disease after P-VABEC chemotherapy. Purpose: To evaluate the activity and toxicity of CIP consolidation therapy after P-VABEC versus a standard P-VABEC regimen in a prospective randomized phase III study. Patients and methods: From October 1995 to June 2000 we enrolled 214 previously untreated pts with diffuse large cell non Hodgkin’s lymphoma (NHL) according to REAL classification, median age 70 yrs (range 60-85), stage II-IV. All eligible pts were randomized at diagnosis to receive P-VABEC (arm A) or P-VABEC-CIP (arm B). The 8 weeks P-VABEC regimen was delivered on out-patient basis as previously described. The CIP regimen consisted of: Cis-platinum (40 mg/td day 1), Idarubicin (15 mg/m2 day 8), and Prednisone (40 mg/td days 1-4/8-11) repeated every 21 days for a total of 3 courses. In the arm B the CIP consolidation therapy was delivered only in pts in CR/PR after P-VABEC. Now 202 patients are evaluable for response, 107 pts randomized for P-VABEC and 95 for P-VABEC-CIP. According to the age-adjusted IPI score 89 pts were considered as Low Risk (IPI 0-1) and 113 as High Risk (IPI 2-3). Results: At a median follow up of 36 months (range 1-88) the CR rate was 72% and 74% (p=ns), the 5-yrs OS was 42% and 61% (p=0.04) and 5-yrs PFS 39% and 51% (p=0.048) respectively in arm A and arm B. According to the IPI score in the Low Risk the 5-yrs OS was 67% vs 70% (p=ns) and 5-yrs PFS was 53% vs 64% (p=ns) while in the High Risk the 5yrs OS was 25% vs 52% (p=0.008) and 5-yrs PFS was 25% vs 36% (p=ns) for Arm A and Arm B respectively. No severe toxicity has been reported for CIP regimen. Conclusions: CIP consolidation chemotherapy, given on out-patients basis after P-VABEC, was a safe and active chemotherapy and improved survival in elderly pts with diffuse large cell lymphoma.

PU046
RITUXIMAB AFTER CHEMOTHERAPY AS FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA
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Background: Rituximab, a chimeric murine/human monoclonal antibody, reacts specifically with the B-cell antigen CD20. The lack of myelotoxicity indicated that rituximab would be well suited for combination therapy with cytotoxic agents. Considering that anthracyclines could increase the complete remission rate in follicular lymphoma, we could assume that the association between CHOP and immunotherapy could increase the number and the duration of complete remissions. Patients and methods: Starting from 1999 we have used Rituximab after chemotherapy as first-line therapy in patients with diagnosis of follicular lymphoma grade I and II according to the REAL classification. Twenty-seven patients after the attainment of complete remission or very good partial remission were treated with Rituximab and they represent the subset of patients analysed. The characteristics of patients were the following: 10 female and 17 male; median age 55 years (range 37-76); 7 patients were in stage II, 10 stage III and 10 stage IV; according to IPI index 15 were low-risk, 11 low-intermediate risk and 1 high-intermediate risk. Bone marrow involvement was present in eight patients (30%); bulky disease was present in three patients; pathologic LDH value in seven patients. All patients were treated with anthracycline containing regimens (CHOP or CHOP-like) and then with Rituximab at the dosage of 375 mg/m2 weekly for four weeks. No grade 4 hematological or extrahaematological toxicity were observed either with chemotherapy or with immunotherapy. Seventeen patients were analysed at the time of diagnosis for bcl2 and seven were positive. All patients were treated as outpatient. Results: After a median follow-up of 31 months (range 8-48) no patients were died. After a median period of 24 months (range 1-41) three patients relapsed; the disease-free survival was 83% at two years. One of the relapsed patients was re-treated with Rituximab and obtained a second complete remission, the other two patients were treated with chemotherapy and also obtained a complete remission. Five out seven bcl2 positive patients obtained a molecular negativization, two after chemotherapy and three after immunotherapy. Two patients were bcl2 positive at the end of therapeutic approach but clinically in complete remission.

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Conclusions: this preliminary study confirm the safety and feasibility of this procedure. The principle aim of our study is to confirm the effect of immunotherapy in the consolidation of the remission. After a median time of 2 years we observed an 83% disease-free survival. Therefore, we can conclude that the use of rituximab could increase the relapse-free survival, although it will be necessary to increase the follow-up time to confirm these data.

PU047
MOLECULAR ANALYSIS OF MENINGEAL INVOLVEMENT IN B-CELL NON-HODGKIN’S LYMPHOMA
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The meninges are a frequent site of localization of hematologic neoplasms including ALL, AML and T Lymphoproliferative diseases, on the contrary is a rare manifestation on B NHL. We report three patients affected of B-NHL with clinical manifestations of meningeal lymphomatosis without parenchymal involvement. M.Z., 30 years affected by B-NHL CD20+ of the stomach with cleaved cells, D.S., 65 years suffering from B-NHL mantle cells CD20+, T.F., 67 years with B-NHL large cells CD20+. All 3 patients suffering from headache, diplopia, lethargy, nausea and vomiting. As this meningeal syndrome progresses papilledema and signs and symptoms of meningeal irritation such as stiff neck and Kerning and Brudzinski signs develop. In the two patients the symptoms appeared after the induction therapy 6 cycles with R-CEOP, in the third patient the clinical sign appeared at admission on Hospital as first diagnosis. In all patient were performed a complete staging, with biochemical analysis, TAC, Bone marrow biopsy, immunophenotypic characterization and RM of SNC and lumbar puncture. The molecular diagnosis of B-cell non Hodgkin Lymphoma is based on determination of clonality of the immunoglobulin heavy chain (IgH) receptor. Analysis of antigen receptor gene rearrangements were performed by polymerase chain reaction (PCR) technique. PCR products are analysed by capillary electrophoresis and laser-induced fluorescence detection. This method has many advantages as good separation and precise sizing of PCR products. Specific gene rearrangement electrophoretic patterns can be identified and recognized in different tissue specimens either synchronous or metachronous in an individual patient. This method was applied to all the patients biopsies. In all 3 patients the same result of molecular analysis was showed. CSF examination revealed the presence of 3500±540 lymphocytes/µL cytomorphologically malignant. Immunoenzymatic analysis showed as B markers. PCR demonstrated one single peak was identified as mononuclelic rearrangements in the first diagnostic collected sample. For every patient sequential samples were tested to compare the clonal patterns: the same rearrangement was found in the different samples; gastric biopsy, ascitic fluid, cerebrospinal fluid, 233bp. In the second patient lymphnode, skin biopsy, cerebrospinal fluid 265 bp in the last patient, lymphnode, cerebrospinal fluid 253 bp, respectively. The same molecular rearrangements in all biopsy and cerebrovascular fluid suggesting that the monoclonal lymphoma clone is responsible of multiple localizations and meningeal infiltration. In all patients treated with methotrexate 15 mg/week as standard dose a complete resolution of meningeal lymphomatosis was demonstrated after 4 weeks after 4 intratetal administration. The diagnosis of meningeal involvement is usually straightforward, but several difficult situations may occur. One such situation arises when meningeal malignancy and infection occur simultaneously. The molecular approach plays a key role in this situation.

PU048
PRIMARY UTERINE NON-HODGKIN’S LYMPHOMA: A REPORT OF THREE CASES
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Primary uterine non-Hodgkin’s lymphoma (NHL) is an extremely rare disease usually involving the cervix rather than the body of the uterus. Most cases are B-cell lymphomas. We report three cases of primary uterine diffuse large B-cell lymphoma (DLBCL) observed during 2002-2003 in our Haematology Division. In all patients lymphoma involved cervix region. Two of these patients were in fertile age: they were 31 years-old (#1) and 34 years-old (#2) respectively and both presented metrorrhaghy as primary symptom. The last patient, a 54 years-old female (#3), suffered for postmenopausal vaginal bleeding and lower abdominal pain. In all cases pelvic examination revealed a cervical mass, confirmed by pelvic ultrasonography. Total-body computed tomography (CT scan) showed an heterogeneous uterine mass without other significant lymphadenopathy or organomegaly. The diagnosis of DLBCL was established by cervical biopsy in all cases. Peripheral blood examination showed only a mild anemia [median Hb value 11 g/dL]. Bone marrow biopsy was essentially unremarkable in the 3 cases, without morphological evidence of involvement by an hematopoetic malignancy. All patients received chemotherapy according to CHOP protocol (cyclophosphamide, adriamycin, vincristine and pred
nism). A radiological re-staging (abdomen and pelvic CT scan and ultrasonography) performed after 3 cycles documented a reduction of uterine mass of 80% in two patients (#2, #3). The other patient was unresponsive and refused further chemotherapy (#1), and she was lost at follow-up. The remaining 2 responsive patients underwent to further 3 CHOP achieving a complete remission (CR). Both performed ring biopsy of uterine cervix (negative for lymphomatous infiltration). Then, as consolidation therapy, the older patient (#3) underwent to radiotherapy (total dose Gy 3960); while the last patient (#2) refused radiotherapy for preservation of procreative function and underwent to other 2 CHOP followed by immunotherapy (4 administration of Rituximab). At present both patients are alive in continuous CR at 9 and 8 months respectively. In our experience primary uterine non-Hodgkin’s lymphoma is a rare event. Because of the rarity of this kind of NHL, the optimal therapy is unknown. Combined chemotherapy and radiotherapy play an important role in the treatment of uterine lymphoma, but immunotherapy could replace radiotherapy as consolidation particularly in young fertile patients to avoid sterility. More studies on large series of patients affected by uterine NHL are needed to identify the most correct therapeutic approach.

PU049
INTRAVENTRICULAR RITUXIMAB COMBINED WITH HIGH DOSE ARA-C IN THE TREATMENT OF RELAPSED MENINGEAL BURKITT’S LEUKEMIA/LYMPHOMA
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Introduction: Relapsed CNS disease during the course of high grade lymphoid malignancies is associated with poor outcome. Intraventricular treatment with the chimeric anti-CD20 monoclonal antibody rituximab has been anecdotally reported as safe and effective in CNS relapsed CD20 positive lymphoma. Case report: A 40 years old male with CD20 positive Burkitt’s leukemia/lymphoma with cerebrospinal fluid (CSF) involvement developed isolated post-radiotherapy meningeal relapse during first line induction therapy according to BFM protocol. After positioning of an Ommaya reservoir, five intraventricular chemotherapy with methotrexate 15 mg, Ara-C 40 mg and dexamethasone were administered with achievement of CNS partial remission defined as continuous persistence of CD20 positive blasts. After obtaining patient’s informed consent, four intraventricular infusions of 25 mg of Rituximab were given with an interval of 2-3 days. The intraventricular injections were well tolerated without any adverse event. During this treatment, CD10/CD20/ CD19 positive blasts gradually decreased in CSF from 5% to 0.31% of the ANC. Two additional courses of high dose Ara-C (24 g/m² total dose) were eventually administered with achievement of immunophenotypic CSF negativity. The patient is still in continuous complete remission, which has now lasted 8 months. Comment: This case suggests the feasibility and the potential usefulness of repeated intraventricular infusions of Rituximab in the management of CSF involvement during the course of CD20 positive high grade lymphomas.

PU050
SEVERE THROMBOCYTOPENIA FOLLOWING RITUXIMAB ADMINISTRATION IN A PATIENT WITH CHRONIC LYMPHOPROLIFERATIVE DISEASE
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Rituximab, a chimeric monoclonal anti-CD20 antibody, is well known for its activity in the treatment of both indolent and aggressive B-cell lymphoid neoplasms. More recently, it has been used in the treatment of antibody-mediated autoimmune diseases such as immune thrombocytopenia, hemolytic anemia and primary cold agglutinin disease, refractory to conventional therapy. At present, only two cases of autoimmune hemolytic anemia (1) and autoimmune thrombocytopenia (2) respectively, have been described, both occurring as consequence of rituximab therapy, in patients with refractory chronic lymphoproliferative diseases. We report the case of a 53-yr-old woman referred to our unit on February 2001, because of moderate leucocytosis (WBC=17.900/mm³) with absolute lymphocytosis and mild splenomegaly. Bone marrow aspiration showed 70% mature small-sized lymphocytes infiltration, associated with a minority of prolymphocytes. Peripheral immunophenotype revealed the positivity of B-cell markers, as well as CD5* and FM C7*. CD23 was negative. Slg pattern was restricted to k isotype. Trephine biopsy confirmed these findings. Therefore, a diagnosis of stage II B-cell lymphocytic leukemia (B-CLL) was made. After six months, as a short lymphocyte doubling time (LDT) was documented (WBC 42.200/mm³, L 85%), therapy with Fludarabine, 25 mg/m²/day x 5 days monthly, for 6 courses, was administered, with achievement of a good partial remission. After 1 year we registered a rapidly progressive disease, with considerable lymphocytosis (WBC 54.400/mm³, L=88%), pronounced splenomegaly (10 cm below costal margin), anemia (Hb 9.6 g/dL) and thrombocytopenia (114.000/mm³). A new bone marrow examination showed the positivity for cyclin D1, suggesting the diagnosis of mantle cell non Hodgkin lymphoma in leukemic phase. Second line therapy with anti-CD 20 monoclonal antibody rituximab (M abthera 375 mg/m²)
on day 1, and CHOP on day 2, was started. Before the first cycle, WBC count was 107,800/mm$^3$, L=95%, Hb 8.3 g/dL, PLT 66,000/mm$^3$. On day 2, leukocyte decrease was associated with drastic fall of platelet count, which increased after five days at pre-treatment level. On following courses this phenomenon occurred similarly, with a transient drop of platelet after rituximab administration. Peripheral lymphocyte phenotype, assessed before and after rituximab therapy, disclosed complete loss of CD20 and CD19, with substantially stable persistence of CD5 and CD19. Clinically, a mild/moderate cutaneous bleeding was observed only after the first rituximab infusion. Likewise to the previously described patient, in this case thrombocytopenia appeared in a lymphoproliferative disease with splenomegaly. The mechanism of platelet destruction is unclear. Perhaps a non-specific phenomenon, such as innocent bystander effect, may be working, enhanced by the concomitant cytokine storm. Nevertheless, an unbalanced equilibrium between different lymphoid and plasmacytoid/plasmacytic clones, which accounts for the therapeutic effect of rituximab in autoimmune cytopenias, could also promote the hegemonic activity of a cellular clone with opposite function and antinomic results.

References


PU051

SYSTEMIC RITUXIMAB THERAPY IN PATIENTS WITH PRIMARY CUTANEOUS B-CELL LYMPHOMAS


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The wide therapeutic use of rituximab, a chimeric monoclonal antibody with murine variable region directed against CD20 antigen and human constant regions IgG1k, is justified by the large diffusion of this transmembrane protein on neoplastic cell surface in B-lymphoproliferative disorders and by important results obtained in their treatment with this monoclonal antibody. We report our experience on the usage of systemic Rituximab therapy in patients with primary cutaneous B-Cell lymphoma (PCBCL). The aim of this study was the evaluation of the efficacy, toxicity and response duration of Rituximab systemic therapy in a rare subset of B-cell lymphoma patients with a good prognosis with traditional treatments. Between February 1999 and April 2003, we treated 18 consecutive patients with a PCBCL (12 males, 6 females), the median age at the time of presentation was 44 years (range 22 to 84). Sixteen patients presented an exclusive cutaneous involvement with either a single (large and deep) lesion or multiple and disseminated lesions. Two patients had a progressive disease with lymph nodes involved. Nine patients were resistant or in relapse after different treatment regimens, 9 patients were untreated. Three patients received Rituximab associated with a chemotherapy (CHT), 1 patient with α-2-interferon (IFN). All patients presented a CD20 positive lymphoma. The histology, according to WHO classification, resulted in a Diffuse Large B-cell Lymphoma (DLCL) in 10 patients, a Marginal Zone Lymphoma (MZL) in 7 patients, a follicular lymphoma in 1 patient. Only 1 patient, had a DLCL recognisable in EORTC classification as a Large cell Lymphoma of the leg. After a complete staging of the disease and informed consent obtained, Rituximab was administered at a dose of 375 mg/m$^2$ intravenously once a week for a total of four (11 patients) or six (7 patients) weekly infusions given as outpatients. None of the patients presented adverse effects during the Rituximab infusion. A severe reduction of circulating B-lymphocytes was observed for 8 months, on the average, after the last administration but no patients showed an increased risk of infectious disease. All 18 treated patients were evaluated for their response to the treatment. Fourteen out of fifteen patients treated with Rituximab alone responded: 11 with a Complete Responses (CR) and 3 with a Partial Response (PR). The patient with Large-cell lymphoma of the leg was resistant to treatment. All patients treated with CHT obtained a CR. Fifteen patients, who completed the treatment at least six months before this paper, were also evaluated for response duration. At 27 months of median follow-up (range 11 to 52), all patients treated with Rituximab alone and 2 with CHT maintained their CR. Three relapses occurred among patients with a lymphoma of marginal zone origin. One patient in PR after rituximab treatment received IFN and obtained a prolonged continuous CR (26 months). Patients with PCBCL, have a good prognosis and a long survival with traditional therapies (surgery, radiotherapy, IFN) but about 50% relapse and need more aggressive treatments. In our experience, Rituximab was effective and safe both in relapsed and in untreated patients and the median response duration is encouraging. A longer observation of responders as well as controlled studies on a larger number of PCBCL patients could demonstrate the utility of rituximab as a front-line therapy despite its high cost.
**PU052**

**RITUXIMAB AS CONSOLIDATION’S TREATMENT AFTER CHEMOTHERAPY IN PATIENTS WITH B-CELL LOW-GRADE NON-HODGKIN’S LYMPHOMA**

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**Introduction.** Rituximab is a chimeric anti-CD20 monoclonal antibody approved as single agent therapy in relapsed or refractory follicular NHL. Results of several studies evaluating rituximab 375mg/m² weekly for 4 weeks showed objective response (OR) rate ranging from approximately 40 and 60% in relapsed/refractory disease. Encouraging data are also emerging about the use of rituximab in combination with chemotherapy (CT) in previously untreated pts with LG-NHL and there are few data about its use as consolidation after first-line chemotherapy. We evaluated the safety and efficacy of rituximab in LG-NHL pts with partial response (PR) after CT.

**Design and Methods:** 15 consecutive pts with LG-NHL, stage III-IV, in PR after first-line CT (10pts) or second/third-line CT (5pts) were treated with rituximab 375mg/m² weekly for 4 weeks. Median age was 59 (range 43-70) and PS 0-1. 10 pts had a bone-marrow’s (BM) involvement and 6 of these showed a minimal infiltration (10%) in BM biopsy. BM involvement in all the pts was also evaluated by cytofluorometric analysis. CT regimens were CHOP or the combination of fludarabine, cyclophosphamide and mitoxantrone. Response was assessed at 3 months after the end of rituximab and then every 4-6 months.

**Results:** All pts are evaluable for response and toxicity. Eight pts (53%) had complete response (CR) and 3 pts (20%) had PR for an overall response rate of 73%. Four pts remained in stable disease. All the six pts with minimal BM involvement achieved CR. Median time to progression is 11+ months (range 3+-35+) and median response duration is 13+ months (range 1+-29+). 7/8 pts (87.5%) are in continuous CR with a median follow-up of 25 months. The toxicity was minimal, mainly infusion-related and rapidly reversible slowing the infusion. The mean serum immunoglobulin levels remained stable. Conclusion: The use of rituximab as consolidation’s treatment after CT may improve CT-induced remission, increasing CR rate particularly in low tumor burden.

**Conclusion:** The use of rituximab as consolidation’s treatment after CT may improve CT-induced remission, increasing CR rate particularly in low tumor burden. Optimal timing of rituximab in the sequence of therapy for LG-NHL has yet to be clarified.

**PU053**

**COMBINATION OF IDARubicin, ETOpOsIDE, CYTArABINE AND DEXAMETHASONe IN RELAPSED OR REFRACTORY NON HODGKIN’S LYMPHOMA**

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**Introduction:** Different therapies can be used to treat relapsed or refractory non-Hodgkin’s lymphoma (NHL) and these should be based on drugs not included in the front-line chemotherapy. For patients who are not transplant candidates and for heavily pretreated patients, when the treatments generally have a palliative intent, the optimal salvage therapy remains to be defined. We evaluated the toxicity and efficacy of the combination of idarubicin, etoposide, citarabine and dexamethasone in unfavorable lymphoma relapsed or resistant to prior doxorubicin- or mitoxantrone-based regimens. Design and Methods: ten patients (pts) with refractory or relapsed NHL (7 large B cell; 3 follicular) were assessed. All pts had relapsed after or failed to respond to anthracycline-based regimen and six of them had received 2 or more regimes of chemotherapy. Median age was 62 years (range 56-72) and PS 1-2. Treatment was given on an outpatient basis: idarubicin 12 mg/m² ev day 1, etoposide 150 mg/m² ev 2 hours c.i day 1, citarabine 500 mg/m² ev 3 hours c.i day 1 and dexamethasone 20 mg/m² os day 1-5. Pts received preventive treatment with fluconazole, ciprofloxacin and ranitidine during treatment. Response was assessed after 3 cycles and responders continued for up 6 cycles. Results: a total of 43 cycles were delivered. The overall response rate to IVAD was 60% after 3 cycles and three of these pts (50%) achieved a complete remission after 6 cycles. The duration of CRs was 10+, 20+ and 21+ months. One of the 3 pts in PR died of causes unrelated to lymphoma. The OS rate for all pts was 13+ months (range 1+-43+). The main toxicity was hematologic: febrile neutropenia grade 3 occurred in 4 pts and grade 4 in 3 pts; none thrombocytopenia grade 3-4 were observed. Extra-hematologic toxicities were insignificant and none pts died for causes related to therapy. Conclusions: IVAD appears a feasible treatment with acceptable toxicity and interesting rate and duration of response in the salvage and palliative setting of heavily pretreated pts with relapsed or refractory NHL. A larger number of pts should be treated to confirm these preliminary suggestions.
PU054
PRIMARY LYMPHOMA OF THE BLADDER TREATED BY ORAL CHEMOTHERAPY
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Primary lymphoma of the bladder is a very rare disorder and about 100 cases have been reported. In fact it represents less than 1% of the urinary bladder neoplasm and only 0.2% of all cases of extranodal lymphomas in North America. Most of these tumours are low-grade B-cell non Hodgkin’s lymphomas of the mucosa-associated lymphoid tissue (MALT) type and have a good prognosis, and responds to a variety of therapeutic approaches. Cystoscopy and biopsy are needed to make the diagnosis and to monitor the patients during follow-up. We report the only case of primary (B-cell CD20+) low-grade lymphoma of the bladder seen at Hospital Pisa University during the last 10 years. It was detected in a 74 years old women, with a history of recurrent urinary tract infections, consulted for macroscopic hematuria. Diagnosis was made by transurethral resection of the lesions and any other sites of lymphoma involvement was excluded by bone-marrow biopsy and molecular analysis and by total body tomography. This patient was treated only with conservative oral chemotherapy: cyclophosphamide 100 mg daily for 15 days monthly for six months. Its cystoscopic appearance at the diagnosis, during and after therapy documented the complete response to treatment. By now the patient is in complete remission with a 14 months follow-up. This localised NHL may benefit from oral conservative treatment by attaining complete remission, normal organ function and very low toxicity in comparison to other chemotherapy regimens and radiotherapy. This case suggests that primary bladder NHL can be managed by conventional chemotherapy for indolent lymphomas.

PU056
MESENTERIC PANNICULITIS PRESENTING AS A HUGE RETROPERITONEAL MASS. A CASE REPORT
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Mesenteric panniculitis is a rare idiopathic disease of the bowel mesentery, characterized by tumor-like infiltration by chronic inflammatory cells, fat necrosis and fibrosis that cal led to sclerosis. It is characterized by an infiltration of lipid-laden macrophages and associated with variable degrees of inflammation. The inflammation produces a lesion which appears as a mass and normally produces abdominal pain without any other significant signs. The radiological pattern is not typical, but the
diagnosis is aided by a number of elements which are important to recognise. Patients usually present with abdominal pain, fever of unknown origin and a palpable mass. Several treatments have been used, including colchicines, corticosteroids associated or not with immunosuppressants. We present an extremely rare case of a 35 year old female of mesenteric panniculitis of the sigmoid colon, presenting as a huge retroperitoneal mass which initially was mistaken for malignancy and that after was complicated by occlusion of the inferior mesenteric vein. The patient presented with a history of abdominal distension and abdominal mass with pain. Physical examination revealed a firm mass in the lower abdomen. Computed tomography showed that the mass arose from the mesentery, which surrounded the mesenteric vessels. The histological findings revealed mesenteric panniculitis and the angiography showed an occlusion of the inferior mesenteric vein that needed a urgent surgery intervention. After she started corticosteroids and immunosuppressive therapy and her symptoms subsequently disappeared during a period of several weeks. The mass in the lower abdomen gradually diminished in size. This may suggest an autoimmune component in the aetiology of mesenteric panniculitis.

**PU057**

**CD20 NEGATIVE RECURRENCE AFTER RITUXIMAB-BASED TREATMENT OF CD20 POSITIVE POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD): A CASE REPORT**


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Occurrence of PTLD is a known complication of long-term immunosuppressive regimes employed to prevent graft rejection. These disorders are usually of B cell origin (over 90% of cases) and EBV-related (70-90% of cases). Optimal first line treatment has not yet been established. Several reports demonstrated the efficacy of the anti-CD20 monoclonal antibody Rituximab. Here we describe a CD20 positive PTLD patient who relapsed with a CD20 negative PTLD after treatment with Rituximab; second complete remission (CR) was achieved with radiotherapy (RT). Case report. This 62 yrs old male patient developed EBER+, CD20+ polyclonal polymorphic hyperplasia PTLD 12 yrs after receiving a heart transplant. At diagnosis, in July 2001, ECOG 1, stage I (right axillary nodes) asymptomatic disease was present; bone marrow was hypoplastic but no lymphocytic infiltrate was demonstrated. Because of concomitant iatrogenic renal failure, he received low dose chemotherapy (CT) with cyclophosphamide 400 mg/day i.v. for 2 consecutive days every 4 weeks, associated with low dose acyclovir (10 mg/kg/day p.o.). Partial remission was obtained after 6 courses of CT. Starting in March 2001 he received 4 additional courses of therapy consisting of rituximab 375 mg/m² i.v. day 1 followed by cyclophosphamide 200 mg/m² i.v./day 2-4 every 4 weeks achieving a CR which lasted approximately 8 months. In January 2003, the patient experienced disease recurrence limited to the right axillary nodes. Node biopsy was consistent with a diagnosis of EBER+, CD20-, polymorphic Hodgkin-like PTLD; clonality could not be assessed. Immunohistochemistry studies were performed on paraffin-embedded tissue sections stained using the Dako anti-CD20, clone L26 and the streptavidin-alkaline phosphatase method according to standard methods. The patient received local RT (40 Gy) and is in CR at 3 month follow up. Discussion. Several studies have validated the use of the anti-CD20 monoclonal antibody Rituximab in patients with some subsets of CD20+ B-cell NHL (e.g. follicular lymphoma, diffuse large cell lymphoma). Similar efficacy and favorable low toxicity profile have been reported in allograft recipients developing PTLD and studies are currently ongoing to test Rituximab as single agent immunotherapy in these patients in whom chemotherapy-related side effects are of special concern. However, as already reported with other forms of immunotherapy (e.g. idiotypic antibody therapy), antigen-negative disease may emerge following immunotherapy with the anti-CD20 antibody. A few cases have already been reported among immunocompetent lymphoma patients treated with Rituximab, but the true prevalence of this phenomenon, the pathogenetic mechanism(s) underlying it (selection of CD20- clones is one possibility) and its clinical impact have not been fully elucidated as yet. To our knowledge, this is the first reported case of CD20- disease at relapse in an allograft recipient treated with combined immunotherapy for a CD20+ PTLD. This case report underlines the importance of performing node biopsy whenever progression or relapse of PTLD is suspected on clinical grounds and highlights the importance of immunophenotyping in addition to morphology in the follow-up of PTLD patients.

**PU058**

**PRIMARY PANCREATIC LYMPHOMA: A FOUR CASES REPORT**


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Primary pancreatic lymphoma (PPL) is an extremely rare disease, representing fewer than 2% of extranodal non-Hodgkin’s lymphomas. We describe four cases of PPL (3 men and 1 woman, mean age 65 years, range
**PU059**

**NASAL NATURAL KILLER CELL LYMPHOMA (LETHAL MIDLINE GRANULOMA): REPORT OF 3 CASES**


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The T-cell/natural killer (NK) cell lymphoma of the nasal cavity and nasopharynx is a recently established, distinctive clinical entity. It has been described as having an aggressive and destructive course characterized by progressive ulceration and necrosis limited to the midfacial region; hence the name lethal midline granuloma. The pathogenesis is likely to be complex. Nasal T/NK cell lymphoma has a characteristic immunophenotype: CD2-positive, CD56-positive, but usually negative for surface CD3. This group of diseases is rare in the United States and Europe but is more common in Asia and Central America. We report 3 cases of males [median age of 40 years-old (range 20-59)] affected by lethal midline granuloma observed in our Hematology Division between 2000-2003. Rhinorrhea, nasal obstruction, rhinolalia and headache were the most frequent presenting symptoms. At observation an ulcer in the middle of the anterior palate in all cases was present. The biopsy specimens taken from palatal lesions revealed the same histologic feature consisting of atypical, small- to medium-sized lymphocytes with irregularly shaped nuclei. Immunohistochemical study showed that these abnormal lymphoid cells were positive for: CD21, CD3 (Leu 4)2, CD3e1, CD43, CD52, CD152, CD56, and CD79a2. The diagnosis of natural-killer (NK) cell lymphoma was established. Radiological procedures (CT scan, MNR) showed the presence of a mass destroying the anterior palate, nasal septum obliterating the maxillary sinuses. No other significant lymphadenopathy or organomegaly were observed at total body-CT scan examination. Bone marrow biopsy did not show an involvement by lymphoma in all cases. All patients received as induction treatment chemotherapy (ProMACE-Cytabom in 2 cases and MACOP-B in the other patient). Two patients presented a progression of disease under treatment and were shifted to a second line chemotherapy (CHOP, IEV) without benefits; they started radiotherapy but died for infectious complication during the treatment. The remaining responsive patient underwent to radiotherapy followed by surgical debridement and plastic reconstruction. At present, after 36 months from diagnosis, he is alive in CCR. The midline granuloma syndrome is a mutilating process that progressively destroy the nose, paranasal sinuses and other regions of the midface. Conventional chemotherapy appeared ineffective for the majority of patients. Only combined treatment with radiotherapy and chemotherapy has reported to improve the prognosis in this kind of patients. However innovative treatment modalities are needed to improve outcome.

**PU060**

**L-VAMP REGIMEN PLUS RADIOTHERAPY IN PATIENTS WITH PRIMARY CNS-NON HODGKIN’S LYMPHOMAS**


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Background: Central nervous system (CNS) non Hodgkin’s lymphoma (NHL) is a rare disease in immune-
PU061
BLASTIC NK LYMPHOMA: A CASE REPORT

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NK cell neoplasms are very rare and not unique lymphoproliferative diseases. The authors report a new case of NK lymphoma. In December 2002, a 72-year-old man was admitted to the hospital because of pruritic erythematous plaques on his trunk, face and scalp and bilateral cervical, axillary and inguinal lymphadenopathies. CBC showed a moderate pancytopenia (GB 3,800/mm3; Hb 9 g/dL; PLT 86,000 mm3) with severe neutropenia (N 332 mmc) and no blasts; LDH was increased; serological work up for B and C viral hepatitis and EBV, was negative. Skin biopsy of a lesion showed diffuse perivascular infiltration of monomorphic medium to large size cells with convoluted nuclei and prominent nucleoli, fine nuclear chromatin and little rim of agranular cytoplasm. Blasts were CD 45+, TdT±CD 57+; CD 56+, CD 34+, CD 79A+, CD 3−, CD 117−, CD 1A+, CD 2−; MIB 1/ KI 67 was increased. Bone marrow showed diffuse infiltration of blasts similar to those seen in the skin which were HLA DR+, CD 56+, CD 36+, CD 4+, CD 33+, CD 103+, TdT+. Bone marrow cytogenetic were normal. T cell receptor gene rearrangement study were negative for clonal rearrangement. Spinal tap revealed leukemic involvement of CSF. Whole body CT scan was unremarkable except for bilateral cervical, axillary and inguinal lymphadenopathies. Flow cytometry immunophenotyping of right inguinal lympho-node revealed the presence of CD 56−, DR+, CD 103+ cells. The diagnosis was blastic NK cell lymphoma. He was threatened with COP (Cyclophosphamide 600 mg/m2 iv day 1; vincristine 2 mg iv day 1; prednisone 10 mg po for 5 days - every three weeks); he received intrathecal methotrexate (MTX) 12 mg and intrathecal methylprednisolone (MPDL) 40 mg every week. After 4 cycles of COP and 4 doses of intrathecal MTX + MPDL, skin lesions disappeared. Skin and bone marrow biopsy and CSF exam were all negative for blasts. Whole body CT scan and PET scan were normal. After 3 month the patient is still in complete remission. NH cell neoplasms have been recognized and are now formally included into WHO classification of lymphoid neoplasms. They are uncommon and difficult to diagnose and can be classified according to their state of maturation in: 1) immature: myleoid/NK cell leukemia; blastic NK cell lymphoma; 2) mature: indolent LGL leukemia; aggressive NK cell leukemia; nasal/nasal type NK lymphoma. In our patient COP chemotherapy was very effective and very well tolerated without significant side effects. A multicentric trial would be necessary to establish the best treatment regimes and the utility a maintenance chemotherapy for this type of neoplasms. COP chemotherapy can be an effective and well tolerated treatment in elderly patients. However further observations need to realize the utility of a maintenance treatment.

PU062
TELOMERASE EXPRESSION AND TELOMERE LENGHT IN B LYMPHOPROLIFERATIVE DISEASES

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Background: Telomeres are repetitive non-coding DNA sequences (5’TTAGGG3’ in humans) at the ends of...
linear chromosomes; they serve a variety of purposes including positioning the chromosomes during cell division, maintenance of chromosomal integrity, protection of unique DNA sequences. Small amounts of these chromosomal terminal sequences are lost in replicating cells during S phase because of incomplete DNA replication, a phenomenon known as the end-replication problem. Several studies have indicated that telomere shortening is one of the most important mechanisms utilized by cells to determine their replicative capacity; with each cell division the chromosomal telomeres become shorter until the cells reach the limit of proliferation (the Hayflick limit or mortality stage 1, or M1), enter in senescence and die (‘mortality stage 2’ or M2). Telomere length is maintained by a balance between processes that shorten and lengthen telomeres and the factor allowing cells to keep on dividing without telomere erosion is the enzyme telomerase. Telomerase, is an unusual enzyme that contains both a protein part and an RNA part: the protein part consists of a reverse transcriptase subunit (hTERT: human telomerase reverse transcriptase) and three or more other proteins; the telomere RNA (hTR) is a small molecule then can adopt a secondary and, probably, tertiary structure with a part complementary to the telomere repeats. It is widely accepted that the activation of hTERT is the most important step for the induction of telomerase activity. Cancer cells and immortalized cells have a prolonged replicative life span, maintaining telomere length usually by activation of the enzyme telomerase which catalyses the addition of hexameric repeats to telomeres. Aim: we investigated telomerase activity and the expression of telomerase subunits in blood (PBL) and bone marrow (BM) of patients affected by B lymphoproliferative diseases; we also evaluate telomere length by the Flow-FISH method.

Methods: A total of 25 patients was enrolled in our study: 18 out of 25 were affected by chronic lymphocytic leukemia, 5 were affected by mantle cell lymphoma and 2 by lymphoplasmocytoid lymphoma. The diagnosis was based on clinical, serological, immuno phenotypic, histological and molecular parameters. Twenty subjects, not affected by hematologic malignancies, age-adjusted served as healthy control. Telomere length measurement of PBL and BM cells was performed by the use of Flow-FISH method. Briefly, PBL and BM cells were hybridized with a FITC-conjugated peptide nucleic acid (PNA) probe (DAKO) and the comparison was made with an internal control (the tetraploid 1301 leukemia cell line, a T-cell line with very long telomeres > 25kb). Telomerase activity was evaluated on CHAPS protein extracts subjected to TRAP assay Telomerase PCR ELISA plus kit (Roche Diagnostics). Quantitative detection of telomerase subunits (mRNA hTERT and mRNA hTR) was performed with the LightCycler Instrument (LightCycler System, Roche Diagnostics, Germany) using Telo TAGGG hTERT and hTR Quantification Kits (Roche Diagnostics).

Results: In order to telomere length we found shorter telomeres as compared to the values observed in subjects of the age-matched control group; this shortening did not correlate with expression of hTERT and hTR (mRNA) levels. The hTERT expression was lower in advanced versus early stages of diseases. Discussion: The availability of standardized laboratory methods allow the introduction of telomere length, telomerase activity and expression in the characterization and monitoring of lymphoproliferative diseases with a better identification of advanced versus early stage of the disease.

PJ063
RITUXIMAB IN THE TREATMENT OF HAIRY CELL LEUKEMIA
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Hairy cell leukemia (HCL) is an indolent B cell neoplasm, strongly expressing CD20. Although it is very responsive to purine analogues, many patients (pts) ultimately relapse. Several new therapeutic approaches have been developed to improve response rates and failure free survival. These include recombinant immunotoxins and unlabelled monoclonal antibodies (Rituximab). Rituximab has demonstrated efficacy as monotherapy and in combination with chemotherapy in the treatment of both aggressive and indolent lymphoproliferative disorders such as HCL, Waldenstrom’s macroglobulinemia, Chronic Lymphocytic Leukemia. In the present study we treated 8 HCL patients (median age 58 years) with Rituximab (375 mg/m² weekly for 4 cycles) after cladribine (2-CDA) as induction therapy. All patients were positive for IgH rearrangement at diagnosis. Four partial response, two complete response and 2 no response were obtained after 2-CDA therapy (overall response rate: 75%). Molecular analysis showed persistence of monoclonal IgH rearrangement. Six out of 8 patients were evaluable after Rituximab monotherapy (the remaining two ones are still under treatment). All six pts (even the two no responders to induction treatment) achieved an hematologic complete response. Moreover 2/6 pts had a complete molecular remission after nine and three months respectively. No toxicity was reported. After a median follow-up of 8 months (range 3-13) no relapse was observed. These data confirm that Rituximab was effective in HCL and they suggest it can play an important role in the treatment of minimal residual disease.
CD30 anaplastic large cell lymphomas are a clinical-pathological entity characterized by frequent occurrence in children (40%), highly aggressive clinical course, usually associated with systemic symptoms and diffuse involvement. Despite of this common features, this lymphoma category presents high heterogeneity either in morphologic-immunophenotypic aspects either in clinical features. 50-60% of cases is characterized by expression of a kinase protein defined anaplastic lymphoma kinase ALK derived by the translocation t(2;5). ALK+ lymphomas have better outcome than ALK negative subtype (5 year survival rate of 80% versus 33%) and an high response rate is obtained with aggressive treatment. With this report we want to present two cases of elderly ALCL ALK+ at diagnosis. Both had an early extranodal stage, B symptoms and were treated with two different regimens (CHOP-rituximab, PVABEC). Both patients achieved a complete remission. No important toxic event was reported. A longer follow-up is needed to evaluate the failure-free-survival and the overall survival. Our experience shows that this entity can occur in elderly patients and that in these cases a good response and outcome can be obtained with aggressive regimens such as in younger patients.

PU065
ACTIVITY OF RITUXIMAB IN LOW-GRADE GASTRIC MALT LYMPHOMAS RESISTANT EITHER TO ANTIBIOTIC THERAPY OR CHEMOTHERAPY
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Helicobacter pylori (HPO) eradication is the standard first-line treatment of gastric MALT lymphoma. However, no treatment guidelines exist for the HPO-negative patients and for the antibiotic-resistant cases: chemotherapy, radiotherapy and surgery, alone or in combination, can be considered but there isn’t definitive evidence of their role due to the lack of randomised trial. Promising results have been recently reported with the use of anti-CD20 monoclonal antibody rituximab in marginal zone nodal or extranodal lymphomas. Here we report our experience on 27 (15M/12F) gastric extranodal marginal zone lymphoma (MALT) lymphoma patients resistant either to antibiotic therapy or chemotherapy all treated with rituximab. Treatment consisted of 4 standard (375 mg/m²) weekly doses of rituximab. The median age was 53 years (range 32-80), according to the Lugano classification, 15 patients were stage I, 7 stage II1, 1 stage II2 and 4 stage IV. Eleven patients had HPO-positive gastric MALT lymphoma resistant or relapsing after first-line antibiotic treatment (and subsequent chemotherapy in 5 patients), the remaining 16 patients had HPO-negative lymphoma progressing or relapsing after chemotherapy (n=4), surgery (n=2) antibiotic therapy (n=8), or rituximab (n=2). Twenty-six patients completed the treatment program and were evaluable for response; one patient was lost to follow-up after the second dose. The objective response rate (ORR) was 73% with 11 complete responses (42%) and 9 partial responses (31%); 7 patients (27%) had stable disease. With a relative short follow-up of 9 months (range 1-38), 2 patients relapsed. We evaluated 15 patients at diagnosis for the presence of 18q21 translocation involving the gene MALT1 using a dual color FISH technique on 2micron-thick sections from formalin-fixed and paraffin-embedded gastric biopsies; 6 (40%) resulted FISH positive. Of note, 2 (33%) of them achieved a CR after rituximab therapy. This seems to suggest that rituximab can be active also in patients presenting t(11;18) translocation, but further studies are required to better clarify the prognostic role of the translocation in this setting. The favorable toxicity profile of rituximab was confirmed: most adverse events were of mild to moderate severity with no grade IV toxicity, thus suggesting that single agent rituximab therapy is safe and effective in gastric MALT lymphomas relapsed after antibiotic therapy or chemotherapy.
evaluate the efficacy of such combination therapy on 28 patients (10 M/18 F, median age 56y (range 30-74), status: I, II, III 7, and IV 12) affected by GI-G2 follicular B-cell NHL. Sixteen patients were newly diagnosed and 12 relapsed; the latter had received almost 2 line of chemotherapy (range 1-7) including ABMT in 2 cases and rituximab in 4 cases. Therapy consisted of Chl 6mg/m² daily for 6 consecutive weeks in association with a standard 4-weekly rituximab administration schedule in the induction phase. After revaluation, patients responding to therapy received 4 additionally cycles with Chl (6mg/m² daily for 2 weeks monthly) plus rituximab (once/monthly). Only one patient had PD so that 27/28 showed a response to combination therapy. Of note 13/16 (81%) newly diagnosed patients obtained CR and all patients PCR BCL-2 positive on peripheral or bone marrow at diagnosis (n=8) become BCL-2 negative after induction and are still negative and in CR at last follow-up. Among 12 pre-treated patients, 4 (33%) showed a CR with BCL-2 negativity. With a median follow-up of 24 months only one patient had a progressive disease. The relatively short follow-up doesn’t justify any definitive conclusion about the duration of response. However the high CR rate observed make such combination therapy suitable as possible first-line alternative treatment.

PU067
IS ENDOSCOPIC ULTRASOUND THE MORE ACCURATE PROCEDURE FOR THE POST-THERAPY EVALUATION OF PRIMARY GASTRIC LYMPHOMA?
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The gastrointestinal (GI) tract is the most common site for primary extranodal lymphomas. Better informations on biology of gastric lymphoma have been associated with a better definition of a diagnostic procedures and strategies of treatment. In this perspective, endoscopic ultrasound (EUS) has been demonstrated as a valuable tool for a correct definition of stage at presentation, but little is known on the role of EUS in the evaluation of response to treatment. On this purpose we conducted a retrospective study to determine the role of endoscopic biopsies (EBx) and EUS in the follow up periodicity of patients with gastric lymphoma. This study included 22 patients with gastric lymphoma divided into two groups: Mucosa Associated Lymphoid Tissue (MALT) Lymphoma (11 pts) and High Grade Lymphoma (11 pts). There were 14 males and 8 females with a median age of 54 years (range 26-80 years). All patients at diagnosis underwent the standard diagnostic evaluation including CT scan, bone marrow biopsy, EBx and EUS. Disease staging was done according to revised Musshoff modification of the Ann Arbor classification system. Three patients were treated with eradication therapy alone; ten received chemotherapy; eight patients received both modalities and one patient received chemotherapy plus radiotherapy. All patients, on completion of lymphoma treatment, were followed with EBx and EUS every three-six months. We found that in 9/11 patients affected by High Grade lymphoma, EUS and EBx showed the same response, while in two cases EUS showed persistence of disease but no significant histologic findings of residual lymphoma was documented by EBx. As regard MALT type, concordant findings of histology and EUS were found through the entire follow up period in only 2 patients; in the remaining 9 patients, in a total of 38 follow up evaluations were performed (with concomitant EBx and EUS); concordant findings of histology and EUS were found in 21 evaluations, while discordant findings were recorded in the remaining 17. In only 3 occasions EUS showed a complete remission while histology revealed residual lymphoma, on the contrary in 14 evaluations EUS showed persistence of disease, but EBx with histology was negative. After a mean follow up period of 36 months we have not observed any relapse in the 9 patients with endosonographic but normal histology. Conclusions. EUS constitutes an useful and reliable methods for local staging of gastric non Hodgkin lymphomas and provides more informations about type and grade of stomach wall infiltration and perigastric lymphonodes involvement. However the impact of this procedure in the follow up of our patients was of limited value. Although our follow up period of 36 months cannot rule out late relapse, our results demonstrate that the abnormalities of gastric wall detected by EUS were not useful to predict relapse in MALT lymphoma. Cumulative data of our analysis suggest the discrepancy of results between EBx and EUS was higher for patients with MALT lymphoma than for high grade lymphoma. We conclude that histological evaluation of endoscopic biopsies still represents the more efficient and accurate restaging procedure in follow up of patients with gastric lymphoma, especially of MALT type.

PU068
THE ROLE OF ANTI HEPATITIS C VIRUS TREATMENT IN HCV-RELATED B-CELL NON HODGKIN LYMPHOMA
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We have previously shown an epidemiological link between HCV infection and B-cell NHL in our geo-
PU069
A LYMPH NODAL ATYPICAL IMMUNE RESPONSE: A CASE REPORT WITH MISLEADING FEATURES
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Graphical area. Indeed, some biological observations so far may indirectly suggest a link between virus and lymphomagenesis, such as the ability of lymphomatous cells to bind viral E2 envelope protein. Furthermore, recently, antiviral therapy against HCV revealed to be efficacious also against HCV-related splenic lymphoma with villous lymphocytes. In January 2001 we planned to test the effect of antiviral therapy on HCV-related indolent low grade B-cell lymphoma (according to REAL classification) both at diagnosis and at relapse. Patients were requested to have well measurable nodal or extranodal disease, indolent course of their disease. During the study no concomitant chemotherapy was allowed. Treatment consisted of pegylated α-interferon 50 µg once a week and daily ribavirin 1 g a day. Up to now, 6 patients entered the study: 5 females and 1 male, mean age 56.8±11.8 years old. Two patients were affected by marginal nodal lymphoma, 2 by lymphoplasmocytoid lymphoma, one by follicular lymphoma and one by splenic marginal zone lymphoma. Three patients were enrolled at diagnosis, while two at first relapse and one at third relapse. In 3 patients the diagnosis of HCV infection preceded by several years the appearance of lymphoma. In only one patient HCV infection caused an increase value of transaminase (3 times normal value). In five, among 6, lymphoma was stage IV disease because bone marrow involvement. In all the 6 patients viral load was over 10×1,000,000/L copies. One patients developed grade IV hemolytic anemia so the treatment had to be stopped an this patient was not evaluated for response. Among the other 5 patients, four showed the disappearance of viral load as well as of the lymphoma (2 marginal zone lymphoma, 1 follicular lymphoma in third relapse and 1 plasmocytoid lymphoma). Their complete response now has been lasting respectively 23, 17, 11 and 8 months. The patient affected by splenic villous lymphoma showed neither decrease in viral load nor clinical response. The strict correlation between disappearance of viral infection and lymphoma strongly suggests a link between HCV infection and HCV-related NHL. Moreover this experience suggests the utility of this kind of therapeutic approach in this subset of patients.

Here we described a reactive, self-limited lymphadenopathy that could easily be mistaken for a malignant lymphoma. Case report: a 32-year-old woman, affected by Sjogren syndrome, presented weight loss, marked asthenia, malaise, and multiple cervical and axillary lymphadenopathy, all less than 2 cm in size. Biochemical investigation showed leukopenia and neutropenia with a moderate anemia and thrombocytopenia and an increased erythrocyte sedimentation rate. Serological tests for Hepatitis A, B, C, HIV 1 and HIV 2 were negative. Rheuma test, nuclear antibody (ANA) and ENA (SSA) were positive. An axillary lymph node biopsy showed a massive proliferation of CD8 positive T immunoblasts that was initially interpreted as a T-cell high aggressive lymphoma. Subsequently, the patient developed septic fever with multiple blood cultures positive for Staphylococcus Epidermidis as well as a Disseminated Intravascular Coagulation (DIC) syndrome. A computed tomography scan of the chest revealed the typical features of acute respiratory distress syndrome (ARDS) and she was admitted to an intensive care unit. Culture examination of bronchoalveolar lavage (BAL) fluid revealed the presence of an Aspergillus and, by polymerase chain reaction, sequences of HHV-6. After intensive antibiotic, antiviral and antimicotic treatment, resolution of ARDS and sepsis occur and the multiple lymphadenopathies spontaneously disappeared within about two months. The patients was than subjected to a further biopsy of a small axillary lymph node. The second histologic examination showed a absence of the immunoblastic proliferation and pictures of an aspecific lymphadenitis. A revaluation of the first biopptic specimen showed the absence of T-cell clonality. Moreover, a thorough immunohistochemical analysis showed the presence of a histiocytic component formed by confluent sheets of CD1a+ elements with only few classic CD68+ macrophages, substantially negative for myeloperoxidase expression. At the best of our knowledge, this case showed clinical, pathological and immunohistochemical features not yet described in literature. However, among the many types of abnormal immune reaction with a well known histological pattern, this case might be considered similar to Kikuchi’s disease and in particular to its proliferative variant characterised by a massive T immunoblastic CD8+ reaction. Very peculiar is the lack of a myeloperoxidase-positive macrophages reaction and a huge expansion of an immunophenotypically Langerhans cell-like population.
Introduction. Primary B cell-NHL (mostly high-grade) arising in the uterus represents a rare but known cause of metrorrhagia; here we describe an hyper-immune reaction occurring in the endometrium of a young patient with abnormal uterine bleeding, which was initially confused with an high-grade B cell-NHL. Case report. An otherwise healthy 31-year-old woman presented with important metrorrhagia and associated post-hemorragic anemia, lasting 5 months and resistant to both contraceptive and hormonal therapies aimed at stopping the bleeding. Following dilatation of the uterine cavity and curettage, an histological diagnosis of large B cell non-Hodgkin lymphoma of the endometrium was made in another institution, based on the finding of a monotonous infiltration in the lamina propria of large lymphoid cells displaying a mature B cell phenotype (CD20+) and a very high proliferation rate (Ki-67 >95%). The patient was then, referred to our centre to receive adequate therapy; a bone marrow biopsy and a total body-magnetic resonance were both normal; laboratory findings (including LDH) were also unremarkable, except for polyclonal iper-gammaglobulinemia and increased IgG levels. Upon careful review of the original slides and additional immuno-histochemical studies, we confirmed the finding of a mature large B cells population massively proliferating in the endometrium, that we further characterized as germinal center-derived (being BCL6+, MUM1−, but its pattern of infiltration (nodules with a meshwork of CD23+ follicular dendritic cells, rather than diffuse effacement), associated to the presence of an accompanying plasma cell population polytypic for the expression of kappa and lambda Ig light chain, prompted us to reconsider the diagnosis of primary endometrial B-DLCL. Indeed, molecular analysis of the IgVH gene rearrangements in the DNA extracted from paraffin sections and PCR-amplified excluded monoclonality of the centroblastic population and unequivocally confirmed that an hyperimmune GC-B cell (and plasma cell) reaction of the endometrium was the cause of the bleeding (and, likely, of the polyclonal hyper-gammaglobulinemia and the increased IgG levels found in the patient’s serum). Serology tests, performed to identify a possible aetiological agent, failed to show signs of active or recent infection by all the pathogens tested (HIV, HBV, HCV, EBV, CMV, VZV, HSV I and II, Toxoplasma). Instead, the therapeutic modalities initially considered (chemotherapy, radiotherapy, hysterectomy with bilateral salpingo-oophorectomy), a watchful waiting approach was undertaken and the young women, who soon recovered normal menses (as well as an histologically normal endometrium), is now alive and well after 1 year of follow-up. Conclusions. To the best of our knowledge, this case represents the first well documented report of an hyper-immune B-cell reaction of the endometrium, closely resembling an high-grade lymphoma, and highlights the pitfalls that can occur in recognizing this reactive disorder and the important therapeutic consequences of its correct diagnosis.
FREQUENT ABERRANT PROMOTER HYPERMETHYLATION OF O6-METHYLGUANINE-DNA METHYLTRANSFERASE AND DEATH ASSOCIATED PROTEIN KINASE GENES IN IMMUNODEFICIENCY-RELATED LYMPHOMAS

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Immune deficiency-related non-Hodgkin lymphoma (NHL) are a heterogeneous group of lymphoid malignancies arising in the context of decreased immune surveillance and comprising i) human immune deficiency virus (HIV)-related NHL; ii) post-transplant lymphoproliferative disorders (PTLD); and iii) NHL complicating primary immune deficiency syndromes, including common variable immunodeficiency (CVI)-related NHL. Aberrant promoter hypermethylation causing gene silencing has been growingly implicated as a mechanism of tumour suppressor gene inactivation in several human cancers. To date, the role of aberrant promoter hypermethylation in the pathogenesis of immunodeficiency-related NHL is not known. These observations prompted our comprehensive analysis by methylation specific PCR aimed at exploring the prevalence of aberrant promoter hypermethylation of O6-methylguanine-DNA methyltransferase (MGMT), death associated protein-kinase (DAP-k), caspase 8 (CASP8) and p73 in 118 immuno-deficiency-related NHL, including 88 HIV-NHL, 25 PTLD and 5 CVI-related NHL. Aberrant hypermethylation of MGMT was detected in 26/79 (32.9%) HIV-NHL, 25 PTLD and 5 CVI-related NHL. Aberrant hypermethylation of DAP-k was detected in 70/84 (83.3%) HIV-NHL, 19/25 (72.0%) PTLD and 3/5 (60.0%) CVI-NHL. Promoter hypermethylation of both MGMT and DAP-k caused loss of the corresponding protein expression when tested by immunohistochemistry or Western blot. Promoter hypermethylation of the p73 and CASP8 genes was rare or absent among immunodeficiency-related NHL. Overall, aberrant promoter hypermethylation in > 1/4 genes was detected in 74/88 (84%) HIV-NHL, 22/25 (88%) PTLD and 4/5 (80%) CVI-NHL and aberrant promoter hypermethylation in > 2/4 genes occurred in 30/88 (34.0%) HIV-NHL, 9/25 (36.0%) PTLD and 2/5 (40.0%) CVI-NHL. The implications of these data are multifold. First, since MGMT is a DNA repair gene, MGMT hypermethylation may be implicated in lymphomagenesis of immunodeficient hosts by favouring genomic instability. Moreover, given its role in predicting resistance to alkylating agents, MGMT hypermethylation may provide prognostic information for immunodeficiency-related lymphomas. Second, promoter hypermethylation of DAP-k represents the most frequent molecular alteration identified to date in immunodeficiency-related lymphomas. DAP-k inactivation results in disruption of the extrinsic pathway of apoptosis initiated by FAS ligand and thus it is likely that DAP-k hypermethylation may represent a major determinant of the FAS resistant phenotype in lymphoma. Moreover, because DAP-k inactivation facilitates c-MYC transformation in vitro, this epigenetic alteration may synergize with c-MYC deregulation in the development of HIV-Burkitt lymphoma. Finally, a fraction of immunodeficiency-related NHL may be characterized by association with the methylator phenotype, that, in other cancers, is regarded as a potential target for molecular therapy.
Cold agglutinin disease (CAD) represents about 10-20% of all cases of autoimmune hemolytic anemia. It is caused by anti-erythrocyte autoantibodies that preferentially bind RBC at low temperatures. CAD can be associated to different conditions, such as B-cell lymphoma, infections (Mycoplasma pneumoniae, Epstein-Barr virus, HIV) and connective inflammatory diseases, or can present as idiopathic disease. Current consensus guidelines for CAD treatment are not available, and therapy is addressed to the underlying disease or to relieve the gravity of symptoms. Idiopathic forms are usually treated with plasmapheresis, prolonged immunosuppression and/or polychemotherapy, often obtaining only transient benefits. We present two cases of CAD in whom rituximab (Mabthera ->) treatment produced a remission that persisted for two years. A 69-year old male was affected by idiopathic CAD for more than 10 years had been treated with different drugs (steroids, danazol, α-interferon) and chemotherapy (chlorambucil, melphalan) without benefits (Hb 7 g/dL, high bilirubin level, splenomegaly, absolute intolerance to cold wheater, high unchanged cold agglutinin titer). Rituximab was administered at weekly dose of 375 mg/m² for four weeks. After treatment, agglutinin titer and spleen volume rapidly decreased, and hemoglobin level increased up to 13 g/dL. This excellent response is still persisting after two years. The second patient is a 60-years old female, affected by concomitant HCV infection and nephropaty who received diagnosis of CAD two years ago. Rituximab, administered at weekly dose of 375 mg/m² for four weeks, induced a progressive and persistent improvement of peripheral blood counts and renal function. In our patients, side effects (chills, fever) were observed only during the first infusion, and were easily controlled by steroids and anti-histaminic drugs. Rituximab seems a promising candidate for first line treatment of cold agglutinin disease.
patients, both in remission for PHL, died: one patient with HCV-related cirrhosis died of hepatorenal syndrome and another died of secondary AM. LHCV-infected patients did not experience major toxic events during the therapy nor had hepatitis reactivation. Thus, HCV infection does not preclude the administration of a chemotherapeutic treatment appropriate for the histological type, and the treatment is effective in inducing CR in the vast majority of patients. The close association over two third of cases of these lymphomas with HCV infection is suggestive of a possible role of this virus in extranodal lymphomagenesis.

PU076
PARADOXICAL BEHAVIOR OF 99mTc-MIBI UPTAKE IN NON-HODGKIN’S LYMPHOMA WITH BCL2 OVER EXPRESSION SOON AFTER CHEMOTHERAPY

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Absent or low 99mTc-MIBI uptake in lymphoma patients is often associated with poor response to the subsequent chemotherapy. We have recently shown that bcl-2 protein overexpression prevents 99mTc-MIBI uptake in malignant tumors (Eur J Nucl Med Mol Imaging 2003, 30:879–87). Interestingly, absent 99mTc-MIBI uptake in bcl-2 overexpressing cell lines was partially restored in the pre-degradative phases of staurosporine-induced apoptosis. These observations raised the possibility that 99mTc-MIBI uptake, assessed in basal condition and in the early phases of drug-induced apoptosis, may be an in vivo surrogate of the assessment of bcl-2 overexpression in lymphoma patients. Aim: To test the effect of standard treatment regimens on 99mTc-MIBI uptake in patients with follicular and large B-cell non-Hodgkin’s lymphoma. Methods: Seven patients were evaluated by 99mTc-MIBI scan prior to any therapy. They were i.v. injected with 740 MBq of 99mTc-MIBI and underwent whole body scan 10 min post-injection. Seven days after the basal scan, patients received the first administration of chemotherapy (CEOP in 5 patients, fludarabine + mitoxantrone in 2); 4 hours after drug administration they underwent a second 99mTc-MIBI scan. Basal and post-treatment scans were then compared. Visualization of each lesion was recorded for each patient, and tumor-to-heart ratios were obtained from both basal and post-treatment scans. Results: Six out of seven patients showed at least one 99mTc-MIBI positive lesion in the pre-treatment scan. Five out of seven patients showed the appearance of additional lesions in the post-treatment scan. An increase of tumor-to-heart ratio, varying between 10% and 60%, was observed post-treatment in all five patients. High levels of bcl-2 were confirmed by immunoperoxidase staining in biopsy specimens obtained from two of two patients tested. In conclusion, these preliminary observations indicate that increased 99mTc-MIBI uptake in lymphoma lesions early after chemotherapy may be related to overexpression of the bcl-2 anti-apoptotic protein.

PU077
THALIDOMIDE AND CYCLOPHOSPHAMIDE IN PATIENTS AFFECTED BY ADVANCED MANTLE CELL LYMPHOMA

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Although mantle cell lymphoma (MCL) has not a very aggressive course, its resistance to chemotherapy makes it one of the worst malignancy among hematologic diseases. Patients affected by MCL often present with disseminated disease and poorly respond to standard chemotherapy or monoclonal antibodies. Better results have been obtained with high dose chemotherapy but this approach is confined to young patients. Recently, a case of MCL responding to thalidomide has been reported (Br J Haematol 119:128, 2002) and we here describe our experience with a palliative treatment using thalidomide and cyclophosphamide in patients with advanced MCL. Our first patient was a 58-year-old male relapsing after a history of treatment with CHOP, splenectomy, HyperCVAD+HdMTX and ARA-C. After last treatment this patient presented with increasing leukocytosis (WBC 40×109/L, with blasts 90%), low platelet count (20×109/L), generalized adenopathies and a palpable abdominal mass. So we decided to treat the patient with supportive treatment (platelet and red cell transfusions) and minimal chemotherapy such as low dose CTX (100 mg/daily orally) but the disease kept progressing. We therefore added thalidomide 200 mg/day and the patient showed a slight but constant improvement of general conditions and blood count and a reduction of lymphoadenopathies so that 9 months after treatment CBC count was normal, physical examination and a CT scan of the abdomen were negative. At the same time a bone marrow biopsy showed the persistence of an infiltration of lymphocytes less than 30% but a peripheral blood immunophenotyping was unable to identify MCL cells. Patient was treated with Thal + CTX for 15 months and therapy has been very well tolerated with only a moderate constipation. However, he developed acute viral hepatitis and died for, while being still in remission for MCL. Second patients was a 66-year-old male who experienced a first relapse after fludarabine+ mitoxantrone and a second relapse after hyperCVAD/Hd M TX-ARA-C. This patient presented CNS and testicular disease other than
a diffuse adenopathy. He was treated with a combination of Thalidomide 200 mg/day and cyclophosphamide 100 mg/day (THAL + CTX) that induced a partial response with reduction of adenopathies and of mediastinal mass. However, after 5 months patient relapsed and died for progression of disease. Third patient was a 60 ys old female with a long history of disease (5 years) and relapses after several combination chemotherapies. After last treatment with hyperCVAD she obtained only a partial remission and the combination of Thalidomide and Cyclophosphamide was started. This therapy induced a further regression of disease and was maintained for 20 months, after that patient relapsed and died of disease. Fourth patient was a 45 ys old male refractory to conventional chemotherpay (CHOP, FND) in whom treatment with thalidomide (200 mg/day) and cyclophosphamide (100 mg/day) induced a significant reduction of a huge abdominal mass that lasted only three months. In conclusion, our experience indicates that the combination of thalidomide and cyclophosphamide has some activity in MCL and it is well tolerated. We think that this combination, together with rituximab, should be explored in elderly patients affected by MCL not eligible to high dose chemotherapy.

**PU078**

**EVALUATION OF EFFICACY AND SAFETY OF PEGYLATED LIPOSOMAL DOXORUBICIN FOR ADVANCED CUTANEOUS T-CELL LYMPHOMA**


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The therapy of advanced Cutaneous T Cell Lymphoma (CTCL) is usually unsatisfactory; no single agent, combination or sequential regimen has clearly demonstrated a real advantage. Pegylated liposomal doxorubicin (PLD) is an anthracycline with a different pharmacokinetic profile and a prolonged half life compared with the free drug doxorubicin; PLD has been approved for use in the treatment of Kaposi’s sarcoma; there is also evidence of a good efficacy and tolerability in patients with various tumors (advanced ovarian cancer, malignant gliomas, advanced breast cancer and non Hodgkin’s lymphomas). Furthermore the results of a recent study suggest that PLD may have a promising role in the treatment of CTCL. The primary outcome measure of our study was the overall response rate (CR+PR); secondary outcome measures were side effects and clinical benefit (i.e. time to treatment failure). Seven patients (2 female, 5 male) aged 29-84 (mean age 64 years) were treated with PLD 20mg/m2 repeated every 28 days for 2-8 cycles; two male patients aged 61 and 80 were treated with a variant of the standard CHOP regimen replacing doxorubicin with PLD 30 mg/m2 (respectively 5 and 6 cycles). Six patients were affected by Mycosis Fungoides (4 patients with stage IB-IIB according to TNM classification, 2 patients with large cell transformation respectively in CTCL CD30+ and CTCL CD30-); two patients were affected by Sézary Syndrome (SS); one patient was affected by primary CTCL CD30+. Pretreatment included: the association α-IFN+PUVA or retinoids and RT (n=2), monochemotherapy (gemcitabine or fludarabine; n=2), polychemotherapy (1-5 lines of treatment also including an autologous stem cell transplant; n=3). The major clinical response was seen after 2-5 cycles (4 CR, 2 very good PR, 1 PR); one patient with SS had a stable disease (SD). The total efficacy rate was the same at the end of the protocol (overall response 88%, 4 CR and 4 PR). The follow-up after the onset of the treatment was 1-26 months (median 7 months). Our results showed that the median OS and PFS were not reached at 12 months; the estimated two-year OS and PFS are respectively 66% and 57%; as for FFTF, after 13 months of treatment 19% of patients were free from events; the reasons for failure were in our study 4 PR, toxicity (one drop out for hypertension) or lack of PR (one patient in SD). Oxycity related to the treatment was accurately monitored; as a whole single agent PLD was successfully tolerated whereas CHOP with PLD was more toxic (grade 4 neutropenia and infectious complications were recorded in 2 patients) in conclusion PLD shows a significant clinical activity and an ad good safety profile (especially as single agent) even in advanced and pretreated CTCL patients.

**PU079**

**MYCOSIS FUNGOIDES/ SEZARY SYNDROME: A REPORT OF THREE CASES TREATED WITH CAMPATH-1H AS SALVAGE TREATMENT**

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We report the use of Alemtuzumab (Campath-1H) as salvage treatment in three patients with advanced mycosis fungoides/ Sézary syndrome who had previ-
ously been treated with conventional chemotherapy. Two patients (case 1 and case 2), aged 42 and 68 years respectively, were heavily pre-treated (>3 prior therapy regimens, including autologous transplant in case 2) and refractory to conventional chemotherapy, and the last patient (case 3), aged 80 years, who had refused any chemotherapy, had been resistant to treatment with cyclosporine and steroids. Campath-1H was administered intravenously, after an escalating dose from 3 to 10 mg, at the dose of 30 mg, three times weekly, to a total dose of 1080, 223 and 480 mg, respectively. The patients with Sézary syndrome (case 2 and case 3) showed clearance of circulating Sézary cells, and clinical improvement of the skin lesions after two weeks of treatment. Two patients (case 1 and case 3) completed the treatment (12 and 6 weeks) without significant toxicity the former achieving a partial response and the latter a clinical complete response. The patient (case 2), who suffered from ischemic cardiopathy and diabetes, quickly achieved a clinical improvement of the Sézary syndrome, but he died because of a myocardial infarction after three weeks of treatment. Our report shows that the treatment with Campath-1H is active even in patients with advanced refractory mycosis fungoide/ Sézary syndrome. Further clinical observations on a larger cohort of patients are needed to establish if Campath-1H may have a role as first line therapy in addition to conventional therapy including chemotherapy.

Hodgkin's Disease

Published Abstracts

HODGKIN'S DISEASE: PREDICTIVE VALUE OF POSITRON EMISSION TOMOGRAPHY PERFORMED AFTER TWO CYCLES OF CHEMOTHERAPY ON TREATMENT OUTCOME. A PRELIMINARY REPORT

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Introduction. Several prognostic models based on simple clinical variables have been proposed for Hodgkin's disease. However, their reproducibility and predictive value when applied in a prospective way is unsatisfactory. Early evaluation of treatment response after a few cycles of chemotherapy by $^{67}$Gallium scan or CT scan has been demonstrated a very useful prognostic tool in Hodgkin's disease. We report here the preliminary results of a multicenter cooperative clinical trial designed to ascertain the prognostic predictive value of PET scan performed early during treatment in patients affected by advanced stage Hodgkin's disease, treated by conventional chemotherapy. Patients and Methods. From January 2002 till now 26 new patients affected by Hodgkin's disease, observed in three Italian Haematological centers, were consecutively enrolled into the trial. All patients underwent a standard baseline staging procedure with CT scan and bone marrow trephine biopsy plus a whole-body PET scan; all the patients were re-staged after two courses of chemotherapy and at the end of the entire therapeutic program, including radiotherapy, by CT scan and PET scan. The interval between the end of the therapy (either chemo- or radiotherapy) and the final restaging was never shorter than 50 days. The clinical characteristics of the patients were as follows: mean age was 31.7 (16-58) years, the male to female ratio was 11/15; 16 patients presented with an advanced disease (stages IIIB through IVB), while 10 patients were in stage IIA. Histopathology was: nodular sclerosis in 21 patients, lymphocyte predominance in 3, mixed cellularity in 1 and lymphocyte depletion in 1. The International Prognostic Score was 0 in 6 pts; 1 in 9; 2 in 5; 3 in 5 and 4 in 1. LDH was abnormal (> 1 × normal values) in 4 pts. Bulky disease was recorded in 8 pts: 4 patients presented extra-nodal disease in one site, 1 in 2 sites and 1 in 4 sites. PET and CT scan revealed an identical disease extent in 24 out of 26 baseline staging procedures while in 2 cases PET was able to upstage disease. Twenty-four patients were treated with ABVD and 2 with escalated BEACOPP regimen. Thirteen out of 26 patients completed the entire program including the final restaging, and are therefore evaluable for the analysis, with a mean follow-up of 115 days (1-260) from the final restaging or progression. After two cycles of chemotherapy CT scan showed partial response in 11 patients and complete response in 4 patients; by contrast, PET was positive in 3 patients and negative in 12. At the end of the program CT scan was negative in all cases. All the patients with a negative PET after two cycles of therapy showed a final PET persistently negative. Two out of three patients with a positive PET scan early during treatment remained positive at the end of the entire program: these two patients are planned to undergo a biopsy in the positive site of the PET scan. The third patient underwent high-dose therapy for progressive disease. Conclusions. Even in absence of a minimum follow-up of one year, we believe that some preliminary conclusions could be drawn: 1. PET scan seems to be equivalent to CT scan in baseline staging of Hodgkin's disease. 2. A positive PET scan performed early during therapy seems to predict an incomplete response to the therapy. 3. Since most of relapses of Hodgkin's disease occur within the first two years after the end of the therapy, definite conclusions will be drawn after a follow up lasting at least the same time.
AN ANALYSIS OF RESPONSE AND SURVIVAL OF 33 PATIENTS WITH ELEVATED HASENCLEVER SCORE IN A GROUP OF 179 PATIENTS AFFECTED BY HODGKIN’S DISEASE

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In the last twenty years the improving of different therapeutic strategies for Hodgkin’s disease has been exemplary. The most important factors that have contributed to this evolution are: strict link between staging methods and related therapies; analysis through clinical trials of new therapeutic programs; better definition of late toxicities; the efforts in order to find reliable evaluation of prognostic factors. Hasenclever (N Engl J Med, 2000) proposed a new International Prognostic Factors Project Score in order to design clinical trials for the treatment of advanced HD and to make therapeutic decisions in single patient. He identified seven factors with similar independent prognostic effects: serum albumin level less than 4gr%; a hemoglobin level of less than 10.5gr%; male sex; age of 45 or older; stage IV disease; leukocytosis (> 15,000/mm³) and lymphocytopenia (lymphocyte < 600 mm³ or < 8% of white-cell count). Patients with HS > 2 are generally considered as a poor prognosis group.

Many studies are ongoing to identify among patients with high HS a group of them with very-poor prognosis to be candidates to receive a more aggressive therapy, including high-dose chemotherapy with autologous or allogeneic bone marrow transplantation. Among our 179 patients affected by HD, for 125 all data required for HS were available: we performed a retrospective study on these patients, who received diagnosis of HD from 1980 to 2000. Most of them received MOPP, ABVD or MOPP/ABVD therapeutic regimens. Twenty patients belong to 0 score (16%), 38 to score 1 (31%), 34 patients are in score 2 (30%); 24 in score 3 (19%); 7 patients belong to score 4 (82%) and 2 patients are in score 5 (2%). At univariate analysis, Hasenclever score (>2), hemoglobin level (<10.5) and stage IV had a significant prognostic influence on both disease-free and overall survival. At multivariate analysis Hasenclever score (>2) was the most important prognostic factor for both disease-free and overall survival. Among 33 patients with HS > 2, 23 achieved a CR; 20 of them are still in CCR after a mean follow-up of 78.5 months; 3 patients relapsed after a mean of 35.4 months. Twenty-five patients are alive with a mean follow-up of 65 months, while 8 patients died after a mean of 22 months.

STEM CELL MOBILIZATION IN NON-RESPONDING OR RELAPSED HODGKIN’ S DISEASE PATIENTS

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Introduction: We report data relative to the mobilization and collection of peripheral blood stem cell in non responding or relapsed HD patients after chemotherapeutic regimens and/or radiotherapy. Statistics: Between 1999 and 2003 we have treated with mobilization regimens and successive collection of PBSC 25 non responding or relapsed patients affected by HD. In 1999 we performed 6 PBSC mobilizations, 7 in 2000, 7 in 2001, 3 in 2002 and 2 in 2003. Median age was 28 (17–57), M/F 10/15. The clinical stage at onset was II in 12 cases, III in 7 cases and IV in 6; bulky disease was present in 10 cases. Histology was MC in 10, NS in 12, PL in 2 and DL in 1. Eighteen patients were at their first relapse (3 months to 15 years), 16 after cycles of ABVD, one after EVE and one after MOPP/ABVD. Another patient first received 14 cycles of COPP, relapsed after 5 years and received 10 cycles of MA-MA; after 10 more years he relapsed again and was submitted to HDS therapy. 4 patients were resistant to ABVD (4–8 cycles) and were consequently submitted to high-doses cyclophosphamide. 9 of the above mentioned patients were previously submitted to extended field Radiotherapy and 3 to autologous stem cell transplantation. We used CTX (7 g/m² + G-CSF 5 µg/kg) in 11 cases, in the remaining 14 cases we have used ifosphamide 3 grams/m² × 4 days, Vinorelbine 25 mg/m² in the first and fifth day + G-CSF at 5. Initial values for CD34+ were 10–494/µL; in two procedures we needed to perform 3 leukapheresis, in 11 procedures we needed to perform 2 and in the remaining 12 procedures only one leukapheresis was needed. We have used the cellular separator Fresenius AS-TEC 204 to process 8–18 liters of blood/leukapheresis. Median PBSC collected in single procedure was 4.87 × 10⁶/kg CD34+ (0.9–21.5) and 3.73 × 10⁴/kg CFU-GM (0–16.7).

Conclusions: We didn’t note differences in failed leukapheresis nor did we note delayed engraftment rates as compared to those in our other patients who underwent autologous transplantation in first line of therapy.
Introduction: elderly Hodgkin's disease (HD) patients (older than 65 years) have a poor prognosis explained in part by age related variables as co-morbidity, in part by the low compliance and the high proportion of toxic events. Few data are available in the literature concerning the best treatment for elderly pts with HD. Patients and methods: we have retrospectively evaluated clinical data of 115 HD patients over 65 years observed in our Institute between 1969 and 2000. Of them, 24 pts (21%) were excluded from our analysis: 21 for insufficient data, 2 because pre-treated on other centre and 1 for death before chemotherapy start. Thus, 91 pts (79%) could be considered for analysis. Patients characteristics at diagnosis and treatment plan are listed in the table. Results. 78 of 91 pts (86%) achieved first CR; 11 of them (14%) relapsed at 2-158 months from first CR (median 28 months) and underwent second line therapy: 8 of 11 relapsed pts died with disease, two are alive with disease and 1 is alive in second CR 5 years from last therapy. 12 of 91 pts (13%) had progression of disease: of them, 8 pts died with disease and 4 pts were lost to follow-up with disease at 12, 13, 19 and 32 months respectively. One out of 91 pts (1%), starting therapy in very bad general conditions, died soon after. No toxic deaths were observed. 12 pts (13%) died in CCR at a median follow-up of 67 months (7-96) for age related causes. In summary, at a median follow-up of 49 months (2-190) 54 pts (59%) are alive in CCR, 1 (1%) is alive in second CR, 7 (8%) are alive with disease and 29 pts (32%) died for disease (17 pts) or for age related causes (12 pts). Conclusions. considering advanced median age (75 years), our results may be considered encouraging and indicating that elderly patients may be cured. In fact, 86% of patients achieved CR and 59% of pts are alive in CCR at a median follow-up of 49 months. We would underline that old patients in good performance status can be cured with good results if they are treated with chemotherapy standard doses (dose intensity); in fact, we observed a poor outcome in this group of patients when they receive an incomplete treatment or a less aggressive one either because of co-morbidity or because of the physician’s unwillingness to recommend intensive treatment. On the other hand, specific, low toxicity regimens are needed for the small population of elderly patients with low performance status, ineligible for conventional treatments.

B-lymphocyte stimulator (BLyS) is a novel member of the Tumor Necrosis Factor (TNF) Ligand superfamily, which is important in B cell homeostasis and responsiveness. BLyS may exist either as a type II transmembrane protein either as a soluble protein derived by enzymatic cleavage of the membrane-bound form. The expression of BLyS is mainly restricted to cells of myeloid origin, such as monocytes, macrophages, dendritic cells and neutrophils. In these cells BLyS production is strongly induced by IL-10, IFN-γ and G-CSF. It has been recently demonstrated that Hodgkin and Reed-Sternberg (HRS) cells, which usually comprise less than 1% of HL, represent clonal populations of transformed germinal centre B cells, while the majority of this tumor is composed of a mixed infiltrate of lymphocytes, eosinophils, fibroblasts, macrophages, neutrophils and plasma cells. Furthermore, the abnormal cytokine/chemokine pattern in HL contributes to proliferation of HRS cells and to impaired host immune response against the neoplastic component. Since increased serum levels of IL-10, a well known BLyS inducing cytokine, have been demonstrated in HL, we investigated the levels of sBLyS in 67 serum samples collected from patients with HL at the diagnosis. Patients were staged as follows: stage I=8, stage II=39, stage III=9, stage IV=10. The correlation between sBLyS and clinical and laboratory features are shown in the table below. The upper normal limit of sBLyS in normal subjects was 5000 pg/mL. sBLyS was augmented in 33/67 (49%) cases of HL at diagnosis with median levels significantly increased compared to normal controls. High concentrations of sBLyS correlated with advanced disease (stages IIb-IV), presence of systemic symptoms and bulky mass. Increased sBLyS was also observed in cases with extranodal involvement. These data suggest a possible role of sBLyS in the survival and proliferation of B-derived HRS cells. The relationship between sBLyS and IL-10 in the pathogenesis of HL and their clinical prognostic significance are currently under investigation.
Polymorphisms in xenobiotic genes can modify an individual’s susceptibility to the carcinogenic effect of environmental and pharmacological agents. The role of these enzyme systems in the pathogenesis of lymphomas is not well known. Generally, these enzyme systems are divided in a phase I class to which the activating cytochrome P-450 (CYP) enzymes belong and a phase II family including conjugating enzymes such as the glutathione-S transferases (GSTM1 and GSTT1). We have previously shown an increased frequency of deletions of the GSTT1 gene in patients with Hodgkin’s lymphoma (Clin Cancer Res, in press). GSTT1 deletions were associated with positive prognostic factors such as a limited stage of disease and an erythrocyte sedimentation rate (ESR) of less than 50 mm/h. We now extended our study to polymorphisms in the CYP1A1 gene. CYP1A1 mutations T6235C (m1), A4889G (m2) and C4887A (m4) were characterized by a PCR-RFLP approach. These mutations define the different alleles: CYP1A1*2A, CYP1A1*2B and CYP1A1*4. We studied 90 patients with Hodgkin’s lymphoma (median age 33 years, range 14-71 years; 36 females and 54 males). All but one patient were treated with standard chemotherapy regimens: 47 patients received ABVD, 29 pts a modified Stanford V regimen (substituting 6 mg/m² metchloramine with 650 mg/m² cyclophosphamide), 7 pts an ABVD/M OPP hybrid regimen, 5 pts BEACOPP and 1 pt VEBEP (26-28). In 42 patients, radiotherapy was included for consolidation. In 73 patients, a complete response was achieved after first-line treatment. Using the Fisher’s exact test, we found no associations between CYP1A1 alleles and patient characteristics, including histotype, stage of disease, presence of B-symptoms, bulky disease and abnormal laboratory parameters. As CYP1A1 is also involved in the metabolism of cytotoxic agents, we next asked whether CYP1A1 genotypes were predictive for response to therapy and prognosis. There were no differences in the remission rates, event-free and overall survival when grouping patients according to the different CYP1A1 alleles, while patients with at least one GST deletion (GSTM1− or GSTT1−) had a significant better disease-free survival when compared to those with undeleted GST genes (GSTM1+/GSTT1+) (p=0.012). We next asked whether combined polymorphic changes in both phase I and phase II enzyme genes could modify the response of patients with Hodgkin’s lymphoma to the cytotoxic therapy. The Cox regression analysis did not show any modifying effect of combinations of CYP1A1 allelic variants with deletions of GSTM1 and/or T1 on disease-free, event-free and overall survival. In conclusion, these data support the possible role of GST deletions in Hodgkin’s lymphoma, while no role for CYP1A1 polymorphic changes became evident.

<table>
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<th>n° of cases</th>
<th>Hbs pg/ml Mean ± SEM</th>
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<td>4485 (2656-11956)</td>
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<td>MC</td>
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<td>LP</td>
<td>3</td>
<td>4228 ± 628</td>
<td>5622 (3576-5484)</td>
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*p-value refers to Mann-Whitney U and Kruskal-Wallis tests. § not available for all cases.
ACUTE MYELOID LEUKEMIAS AND MYELODYSPLASTIC SYNDROMES

Acute Myeloid Leukemias

PU086
PROGNOSTIC FACTORS AND EXPRESSION OF MULTIDRUG-RESISTANCE RELATED PROTEINS IN 36 PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA
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We report a single centre experience about the characteristics and outcome of 36 APL patients (pts) observed at our Department of Hematology between 1990 to 2002. The expression of multidrug resistance (MDR) associated proteins (PGP, LRP, MRP1) was also analysed. There were 12 males and 24 females (median age 37 years), 89% (32/36) with classic APL morphology and 11% (4/36) with a microgranular variant. Risk class (according to GIMEMA/PETHEMA): 25% (9/36) High Risk (HR), 53% (19/36) Intermediate Risk (IR), 22% (8/36) Low Risk (LR). PGP, LRP and MRP1 expression at onset and at first relapse was low. CD33 antigen expression was high in all cases. The pts were treated according to GIMEMA protocols (LAP0493 and AIDA) including ATRA in induction in 75% (27/36) of cases; 94% (34/36) of pts achieved a CR after induction therapy while 6% (2/36) died early (DDI) of hemorrhage. Outcome: 71% (24/34) of evaluable pts remain in CR at a median follow-up of 57 months (range 4-158) while 29% (10/36) relapsed at a median time of 12 months (range 8-43) and of them 8/10 died early. The majority of pts that relapsed were in HR group. The OS of the whole population at 32 months was 66% and the DFS at 42 months was 62%. A statistically significant difference in terms of DFS was observed between HR and IR/LR pts (p 0.04 by log-rank). DFS was not affected by age, sex, HB levels, karyotype, BCR isoform. At conclusion our data confirm that despite the high rate of success with ATRA plus chemotherapy as induction (more than 90% of CR), about 30% of APL patients have a relapse (without a long lasting second remission) and underline the importance of patient stratification in distinct risk-groups at diagnosis in order to better adapt the kind and intensity of treatment. Taking in account the high expression of CD33 and the low expression of MDR proteins in APL, new and investigational approaches such as gemtuzumab-ozogamicin, with or without ATRA and other new drugs, should be considered, especially in HR APL patients.

PU087
MOLECULAR REMISSION IN REFRACTORY ACUTE PROMYELOCYTIC LEUKEMIA FOLLOWING GEMTUZUMAB OZOGAMICIN TREATMENT. A CASE REPORT
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A 36-year-old man was admitted on 25 November 2002 with a history of fever, right upper quadrant abdominal pain and vomiting. Laboratory tests showed 11×10⁹/L leukocytes (WBC) with 64% of blasts, 59×10⁹/L platelets, hemoglobin 90 g/dL, prolongation of thromboplastin and prothrombin time, increased level of fibrin degradation products and hypofibrinogenemia. Bone marrow (BM) aspirate disclosed 100% leukemic infiltration by blasts with irregular, folded or bilobated nucleus and cytoplasm containing a few azurophilic granules and occasionally Auer rods. The flow cytometry immunophenotyping was positive for myeloperoxidase, CD9, CD13, CD33, CD117 and negative for HLA-DR and CD34. Karyotypic studies failed, but polymerase chain reaction (PCR) testing at a level of 10⁻⁴ showed PML/RAR α rearrangement (bcr1 isoform type), thus confirming the diagnosis of microgranular variant of acute promyelocytic leukemia (APL M3y). Abdominal pain worsened and was followed by the rapid onset of hepatomegaly, weight gain, ascites with elevation of levels of transaminases, bilirubin, γ glutamyl transferase (GGT) and alkaline phosphatases (ALP). An abdominal ultrasonography study revealed dishomogeneous hepatomegaly with portal and hepatic vein thrombosis. The patient was given combined induction treatment with all-trans retinoic acid (45 mg/m²/daily) and idarubicin (IDA)(12 mg/m² on days 2, 4 and 6) according to AIDA protocol, associated with sodic heparin anticoagulation. The induction therapy was discontinued after 6 days because of the increase of WBC up to 55×10⁹/L with 64% of blasts; thereafter he was given two courses of cytarabine (ARA-C) 1 g/m² daily for 4 days with an interval of 14 days, with improvement of coagulopathy but persistence of leukocytoses, bilirubin, γ glutamyl transference (GGT) and alkaline phosphatases (ALP). An abdominal ultrasonography study revealed dishomogeneous hepatomegaly with portal and hepatic vein thrombosis. The patient was given combined induction treatment with all-trans retinoic acid (45 mg/m²/daily) and idarubicin (IDA)(12 mg/m² on days 2, 4 and 6) according to AIDA protocol, associated with sodic heparin anticoagulation. The induction therapy was discontinued after 6 days because of the increase of WBC up to 55×10⁹/L with 64% of blasts; thereafter he was given two courses of cytarabine (ARA-C) 1 g/m² daily for 4 days with an interval of 14 days, with improvement of coagulopathy but persistence of leukocytoses and thrombocytopenia. At this time, BM aspirate disclosed 90% leukemic infiltration. On 10 December 2002 he complained an acute and worsening left upper quadrant abdominal pain, therefore he underwent surgical examination with splenectomy because of partial splenic rupture. Three days later a transjugular intrahepatic porta-systemic shunt (TIPS) was placed under fluoroscopic guidance with lowering of portal pressure measurement from 35 until 25 mmHg. At this time anticoagulation treatment with sodic heparin was withdrawn and substituted by low molecular weight heparin. Thereafter ascites slowly improved, serum levels of bilirubin and transaminases became normal in a few weeks, but a persistent fivefold elevation of GGT and ALP was recorded. After the achievement of informed consent, on 21 December 2002 he was given therapy with
gemtuzumab ozogamicin (Mylotarg®) 9 mg/m²; infusion-related toxicity was mild and included chills and hypoxemia. At this time, a course of lipid complex amphotericin B treatment, at a dosage of 5 mg/kg daily until a total dose of 5 g, was administered for probable invasive pulmonary aspergillosis (IPA) according to the EORTC/MSG criteria. On 09 January 2003 he achieved complete remission (CR) but PCR turned out to be negative for PM/L/RARα. The persistent elevation of GGT and ALP prompted to liver biopsy in January 2003, histologic examination showed very mild centrilobular necrosis and cholestasis. Thereafter, on 01 February 2003 a second course of Mylotarg® 9 mg/m² was given without relapse and cholestasis. Thereafter, on 01 February 2003 a second course of Mylotarg® was given after the same dose was administered for 15 days every 4 months. Molecular follow up performed on 11 April 2003 turned out to be persistently negative. The CR rate was 16/19 (84%) and patients in CR achieved PCR negativity in 11 out of 22 APL patients in CR but still PCR positive for PM/L/RARα; after induction treatment with ATRA with or without chemotherapy. Mylotarg® is an engineered human anti-CD33 antibody (hp 67.6) conjugated with the cytotoxic antibiotic calicheamicin; the latter, given its similarities with antaclycines; appears to be a very promising drug in treatment of APL patients. Scattered reports of Mylotarg® treatment in APL patients, both untreated or at the time of relapse, have been reported. Combined induction treatment with a single dose of Mylotarg® 9 mg/m² and ATRA (45 mg/m²/daily) was given in 19 untreated APL patients. The CR rate was 16/19 (84%) and patients in CR received further courses (up to 8) of Mylotarg® and ATRA. 14 out of 16 patients have become PCR-negative, including 6 who became negative after induction, and 2 after 1 post-CR course, respectively, none of them have reverted to PCR positivity. A prolonged hematologic and molecular remission for 11 months have been already reported in a patient with advanced APL after 2 doses of Mylotarg® at standard dose, given at the time of the third relapse. Mylotarg® appears highly active in APL, both at diagnosis or at the time of relapse, being able to induce R Mol in one ATRA/chemotherapy refractory patient even after only two doses and its administration was feasible even in presence of hepatotoxicity. A randomized trial might address the comparative benefits of Mylotarg® treatment in relapsed or refractory patients. This approach might spare potentially curable patients long term toxicity.

CD38 is 45 KDa transmembrane glycoprotein that is widely expressed on hematologic and non-hematologic cells. CD38 is involved in many different pathways of differentiation, proliferation and maturation of hematologic cells. It is present on a subset of more maturing stem cells (CD34+, CD34-). Its clinical significance is still unclear in hematologic malignancies. It has been correlated with a poor prognosis in chronic lymphocytic leukemias whereas in acute myeloid leukemias seems to be related with a favorable prognosis (Keyhani A, Leukemia Research, 1999). We investigated 33 acute myeloid leukemias (26 de novo and 7 secondary to myelodysplasia) in order to evaluate its expression and its association with CD34. CD38 was expressed on all (26/33) de novo acute myeloid leukemias but it was not expressed on 7/33 secondary to myelodysplasia acute myeloid leukemias. CD34 was present on all (7/33) secondary acute myeloid leukemias, while its expression was variable on de novo leukemias. According to FAB criteria classification we found two distinct patterns of expression of CD38. In acute promyelocytic leukemias-M3 (5/33), CD34 always negative, CD38 was significatively expressed at low density in all samples tested. Its expression in other FAB subtypes was bright or very bright. Interestingly, in FAB subtypes M5 (6/33) we always observed the presence of CD38 (bright) and the absence of CD34 expression. In the others FAB subtypes no clear association was present when considering co-expression of CD38 and CD34 (9/15 CD34 positive acute leukemias). Data are summarized as follows:

- De novo acute myeloid leukemias (26/33):
  - FAB M 3 (5/26): CD38bright CD34−
  - FAB M 5 (6/26): CD38bright CD34−
  - Other FAB subtypes (15/26): CD38bright CD34v (9/15 CD34+).

Secondary to myelodysplasia acute myeloid leukemias (7/33): CD38− CD34+ (7/7). Further studies are needed to confirm that there are specific patterns of co-expression of CD38 and CD34 in acute promyelocytic leukemias-M3 and in FAB M5 acute myeloid leukemias. On the other hand, our results seems to confirm the more immature origin of blast cells in secondary acute myeloid leukemias evolving from myelodysplastic syndromes.
PU089
CHEMOTHERAPY IN REFRACTORY ACUTE MYELOID LEUKEMIA AND PULMONARY ASPERGILLOSIS: FEASIBILITY IN TWO CASES USING VORICONAZOLE
UO di Ematologia, *Servizio di radiologia, PO San Gennaro, ASL Napoli 1, Italy

Aspergillus is one of the most serious complications in leukemic patients treated with aggressive chemotherapy. It is life-threatening with a high mortality rate (40-50%). Complete response to antifungal therapy is reported with Amphotericin-B in about 57% of patients. One of the most important risk factors for aspergillosis is neutropenia, and eradication of the infection depends on the number of neutrophils. So consolidation therapy and/or transplant, that could be at risk of reactivation of aspergillosis foci during aplastic phase, are often delayed if there are still detectable nodules with TC scan or positive cultures. This is in complete remission patients for leukemic disease. More difficult and very disappointed is to treat refractory patients to antifungal therapy and affected by active leukemia. Here we report two cases of resistant patients for leukemia and fungus, in whom salvage chemotherapy was not delayed, well tolerated, it was possible to reach complete remission of the leukemic disease and aspergillosis was responsive using voriconazole. Case 1: DLR, male, aged 58, received diagnosis of acute myeloid leukemia FAB M4 in september 2002. Patient was enrolled in the GIMEMA LAM 99p protocol. At day +14 developed fever, and at day +18 chest-x ray showed lung infiltration. For FUO empirical antifungal therapy with Ambisome 5 mg/kg was beginned togheter to G-CSF. At day +30 bone marrow showed resistant leukemic disease. At TC scan multiple lesions within 1 and 4 cm in both lungs were detectable. Patient was still febrile and sputum culture was positive for Aspergillus Fumigatus. At day +50 endovenous voriconazole 4 mg/day treatment was started for 14 days, then orally. At this time, second induction according to GIMEMA LAM99p was began. During aplasia only two days of fever due to a klebsiella sepsi were present. No other systemic complication was experienced by the patient. Bone marrow at recovery showed complete remission of the leukemic disease, TC scan resulted in a decrease of the diameter of the lung nodule but the appearence of two little new localitation in apical right lung. Consolidation therapy was started at day +50. It was well tolerated, without febrile episodes or systemic complications. TC scan performed at the end of the consolidation cycle showed complete remission of the lung infiltrates. Because of the presence of HLA-identical sibling patient is actually undergoing allogeneic transplantation.

PU090
NOT ELIGIBLE ACUTE LEUKEMIAS: ONE YEAR EXPERIENCE IN A SINGLE CENTER
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During the last year 22 newly diagnosed acute leukemias were reffered to our institution. 19 were myeloid and 3 lymphoid. 11/22 (50%) were not eligible for national protocols of cooperative groups to which our center joins. We wanted to retrospectly asses the main characteristic, treatment and outcome of this kind of patients (Table 1). The non eligibiliti was due to age (6), active infection (2), pregress cancer (1), hepatic failure (1) and one because protocol was not yet active in our center for burocratic problems. Of this 11 patients, 1 died first any therapy could be instaured, of the remaining 10, 9 reached CR, and 5 are still alive in CCR. Two patients died for non-hematologic causes. Data about patients that are not enrolled in any protocol for every reason are not available. They are rarely recorded, and our experience, even if in a small num-
ber and non homogeneous patients, suggests some interest to collect this data in larger series, in order to design specific protocols for the therapy of non eligibles patients.

Table 1.

<table>
<thead>
<tr>
<th>Age/sex</th>
<th>FAB</th>
<th>Karyotype</th>
<th>Treatment</th>
<th>R.C.</th>
<th>Relapse</th>
<th>Status</th>
<th>Follow-up/months</th>
</tr>
</thead>
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<tr>
<td>1 76/F</td>
<td>AML/M2</td>
<td>normal</td>
<td>Dnr 1-3</td>
<td>No</td>
<td>—</td>
<td>dead</td>
<td>3 +</td>
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<tr>
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<td>Ara-C 1-7</td>
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<td>8 +</td>
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<td>Ara-C 1-7</td>
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<tr>
<td>4 70/M</td>
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<td>normal</td>
<td>VP16 1-3</td>
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<td>no</td>
<td>dead*</td>
<td>4 +</td>
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<tr>
<td>5 68/F</td>
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<td>Ara-C 1-7</td>
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<td>12</td>
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<tr>
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<td>Ara-C 1-7</td>
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<td>4 months</td>
<td>dead</td>
<td>4 +</td>
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<td>7 80/M</td>
<td>AML/M1</td>
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<td>12</td>
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</tr>
<tr>
<td>9 63/M</td>
<td>ALL/L2</td>
<td>complex</td>
<td>VP16 1-3</td>
<td>No</td>
<td>no</td>
<td>alive</td>
<td>11</td>
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<tr>
<td>10 75/F</td>
<td>ALL/L2</td>
<td>normal</td>
<td>Ara-C 1-7</td>
<td>No</td>
<td>alive</td>
<td>11</td>
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<tr>
<td>11 79/F</td>
<td>ALL/L2</td>
<td>(MLL)</td>
<td>VP16 1-3</td>
<td>No</td>
<td>alive</td>
<td>11</td>
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</tr>
</tbody>
</table>

*not for hematologic causes.

PU091

BENIGN THYMIC HYPERPLASIA AFTER INDUCTION-CONSOLIDATION THERAPY FOR ACUTE MYELOID LEUKEMIA


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Thymic hyperplasia after recovery from chemotherapy for different malignancies is an uncommon finding described most frequently in patients with solid tumours, while it is extremely rare in patients with acute leukemia. In most cases no symptoms are present, while radiologic examination shows typically a mediastinal mass. The possible cause could be a rebound enlargement after initial atrophy caused by cytotoxic agents. Here we report a case of thymic hyperplasia in a young patient after induction and consolidation chemotherapy for acute myeloid leukemia (AML). On February 2003, a 16-year-old male was diagnosed as having M2-AML, according to FAB classification. Leukocyte count was 184×10^9/L with 98% blasts, hemoglobin level and platelet count were 9.4 g/dL and 29×10^9/L, respectively. The bone marrow aspirate was hypercellular with 90% hypergranular blasts, chromosomal analysis of bone marrow cells showed a normal karyotype. The patient received cytarabine 150 mg/daily for 3 days in order to reduce hyperleukocytosis, and then induction treatment consisting of idarubicin (10 mg/m^2 on days 1, 3, 5), cytarabine (100 mg/m^2 as continuous infusion on days 1 to 7) and etoposide (100 mg/m^2 on days 1 to 4), and complete remission (CR) was achieved after prolonged pancytopenia. Then, according to the protocol, a consolidation course, consisting of cytarabine 500 mg/m^2 q12h from day 1 to 6 plus mitoxantrone 12 mg/m^2 from day 4 to 6 was administered. In addition, the patient underwent CNS prophylaxis with three doses of intrathecal methotrexate. The chest X-rays, performed either at diagnosis or at hematopoietic recovery after consolidation therapy, did not show any abnormality. On May 2003, two months after consolidation while in CR, the patient underwent clinical and radiologic evaluation for allogeneic stem cell transplantation from a HLA identical sibling. The CT scan showed an anterior mediastinal mass, rising consistent doubt among leukemia recurrence as granulocytic sarcoma, lymphoma or thymic hyperplasia. However, chest MRI definitively showed the thymic origin of the mass, consistent with a diagnosis of benign thymic hyperplasia. On this basis, neither biopsy was performed, nor any treatment was started, and the patient was readmitted for allogeneic stem cell transplantation. In the literature, several cases of thymic hyperplasia have been reported, mostly after chemotherapy for solid tumours. On the contrary, sporadic cases have been described in hematologic malignancies, and, at the best of our knowledge, only two cases in acute leukemia. This finding emphasizes that benign thymic hyperplasia should be considered in the differential diagnosis of mediastinal masses in patients with acute leukemia following intensive chemotherapy, in order to avoid needless invasive investigations or overtreatment.

PU092

ISOLATED GRANULOCYTIC SARCOMA OF THE ILEUM IN A PATIENT WITH NORMAL CYTOGENETICS AND MOLECULARLY POSITIVE FOR CBFβ/MYH11 FUSION TRANSCRIPT


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Granulocytic sarcoma (GS), also known as chloroma, is an infrequent extramedullary tumor, composed of immature myeloid cells and, occasionally, of cells with...
a variable degree of granulocytic differentiation. Overall, GS is observed in less than 5% of cases of acute myeloid leukemia (AML) and is preferentially associated with M2 morphology and t(8;21)(q22;q22), while it is extremely rare in AML with inv(16). S.A., a 52 year-old male was admitted to the gastroenterology department of our hospital because of severe abdominal pain and emesis. Following physical examination and CT scan showing a large peritoneal mass, a diagnosis of intestinal obstruction was made and the patient underwent surgery with partial resection of the ileum. Whole body CT scan did not reveal other abnormal findings. Histopathological examination of the surgical specimen demonstrated a diffuse infiltrate of the mucosa consisting of medium to large immature cells, which were stained at immunohistochemistry by the leucocyte marker CD45 and the myeloid markers CD34 and HLA-DR. In addition, cells were strongly positive to anti-myeloperoxidase antibody staining. On this basis, a diagnosis of GS of the ileum was performed and the patient was referred to our institution. At physical examination, neither enlarged lymph nodes, nor hepatosplenomegaly were found. Blood count and differential as well as chemistry were within normal range. Of note, bone marrow morphological examination as well as immunophenotypic analysis did not show any evidence of leukemia; in addition, marrow eosinophilia was not found; finally, cytogenetic examination revealed a normal karyotype (46, XY in 25/25 fully evaluated metaphases), apparently consistent with diagnosis of isolated intestinal GS. However, at molecular evaluation, including investigation of AML1/ETO, DEK/CAN, BCR/ABL, PMI/RARα and CBFβ/MYH11 hybrid fusion transcripts, a clear positivity was found for CBFβ/MYH11. After recovery from surgery, the patient received AML like induction chemotherapy (idarubicin plus cytarabine plus etoposide) and consolidation with intermediate dose cytarabine + mitoxantrone (1gr/m2 on day 1 to 6 and 10 mg/m2 on day 4 to 6, respectively). An additional cycle of high dose cytarabine was administered and, at hematopoietic recovery, 5×10⁶/kg CD34 positive cells were collected; the patient is programmed to receive two further consolidation with high dose cytarabine. To the best of our knowledge, only three reports have described isolated GS in AML with inv(16); however, in all cases, inv(16) was easily detectable at conventional cytogenetic examination and in two of them additional chromosomal abnormalities were present, i.e. trisomy 22. Our case is unique in that the presence of inv(16) was only detectable by the presence of the CBFβ/MYH11 hybrid transcript, which represents the molecular counterpart of inv(16). This finding highlights the importance of investigating at molecular level any patient with apparently isolated granulocytic sarcoma, including those with normal cytogenetics. In fact, while in these cases it is well established that the administration of AML-like chemotherapy results in a significant advantage in terms of delayed AML onset and survival, the detection of molecular abnormalities may contribute to a more correct diagnosis as well as to careful monitoring of therapeutic results.
REMISION OF MYELOID LEUKEMIA M2 WITH GEMTUZUMAB OZOGAMICIN ALONE

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Recently a new approach to hematologic diseases has been introduced using a magic bullet for treating leukemia. Gmtuzumab ozogamicin (GO) is a monoclonal antibody immunoconjugate developed for the treatment of acute myeloid leukemia (AML). Actually, it is possible to use GO for treatment of refractory leukemia in association with standard chemotherapy in AML expressing the CD33 antigen in patients older than 60 years. We report here a case of AML treated with GO alone for hypersensibility to cytosine arabinoside. In June 2002 ZC, a 73 year-old man, was admitted to our hospital with fever, anemia, leukopenia with blast cells and severe thrombocytopenia. The bone marrow analysis and flow cytometric confirmed the diagnosis of acute myeloid leukemia M2 FAB classification. The receptors evaluation of flow cytometric showed a higher expression of CD33 on leukaemic cells, and a co-expression of CD34/CD15. Cytogenetic analysis evidenced chromosomal aberrations with translocations (8;21)q22:q22 and deletion of (9)q22. The patient was treated immediately with GO at the recommended dosage of 9 mg/m², administered as a 2-hour intravenous infusion for a total of 2 doses with 14 days between doses. Complete leukemia clearance was defined as a neutrophil count of 1000/µL or more, platelet count >20,000/µL, no circulating blasts, and bone marrow differential with <5% blasts. We reported the data of complete remission after two infusions of GO on 9 September 2002. The criteria for complete remission has been obtained with the disappearance of blasts, CD33 and CD34/CD15 positive cells and chromosomal aberrations. The adverse effects of GO in our patient are neutropenia, prolonged thrombocytopenia and severe thrombocytopenia. The bone marrow analysis and flow cytometric and cytogenetic analysis and have a good quality of life. Administration of gemtuzumab ozogamicin to patients with CD33+ AML in first diagnosis could induce complete remission. GO treatment appears to have a favorable safety profile particularly in older patients. These new approaches need to undergo further investigation in controlled trials.
Modena, Italy
Ematologia, Università di Modena e Reggio Emilia, Dipartimento di Oncologia ed Ematologia, Sezione di

WITH DEFIBROTIDE FOLLOWING GEMTUZUMAB OZOGAMICIN: SUCCESSFUL TREATMENT LATE OCCURRENCE OF HEPATIC VENO-OCCCLUSIVE DISEASE PU096

Gemtuzumab ozogamicin (GO) (Mylotarg CM A-676) is a novel chemotherapeutic agent consisting of an anti-CD33 monoclonal antibody linked to calicheamicin. The myeloid cell surface antigen CD33 is expressed by the leukemic blasts of at least 90% of acute myeloid leukemia (AML) patients. It has been reported that CD33-positive acute myeloid leukemia patients in first relapse treated with GO achieve a 20-30% response rate. GO therapy is associated with a 20% incidence of grade 3 or 4 hepatotoxicity, and has recently also been associated with hepatic veno-occlusive disease (VOD), a clinical syndrome diagnosed by Seattle and Baltimore standard criteria, including hyperbilirubinemia (bilirubin > 2 mg/dL) accompanied by one or more of the following symptoms: painful hepatomegaly, fluid retention (e.g., ascites), or sudden weight gain (>5% of pre-treatment weight). The median time of occurrence of VOD is 25 days (from 10 to 35 days) after the first infusion of GO. We report the unusual case of a 67 year old female patient with AML, refractory to standard induction therapy, who had developed VOD at day +69 from the start of GO treatment. At the beginning of GO therapy bilirubin was 0.9 mg/dL, while AST and ALT were 24 U/L and 33 U/L, respectively. At discharge, at day +42, after GO therapy, liver enzymes and bilirubin were normal and maintained in normal range until day +69, when the patient was referred again to the hospital for abdominal pain. The physical examination revealed liver enlargement, ascites and weight gain (8 kg in 7 days). Laboratory tests showed hyperbilirubinemia (bilirubin 3.5 mg/dL), without any increase of aminotransferases, grade II leukopenia and grade IV thrombocytopenia. An ultrasound echotomography performed on day +70 confirmed ascites, showing findings highly suggestive of VOD (increased hepatic artery resistance index and decreased portal venous flow). Because of severe thrombocytopenia, the patient was treated with defibrotide at 20 mg/kg/day from day +76 to day +87, without further dose adjustments, achieving a progressive resolution of the clinical picture, with normalization of bilirubin level. At day +89 an ultrasound echotomography showed absence of ascites. Peripheral and bone marrow examinations at day +83 from the infusion of GO, showed a partial complete remission (CRp). The mechanisms of the GO associated VOD are unknown. A direct toxic effect of calicheamicin on the liver has been hypothesized on the basis of the co-expression of CD33 antigen on hepatic sinusoidal endothelial cells, which may be eventually favoured by the use of higher doses of the immunoconjugate. The atypical late occurrence of VOD in this patient suggest the need for a close monitoring of AML patients treated with GO, even for weeks after the first month from the start of therapy. The successful outcome after low dose defibrotide in this patient supports this therapeutic option in the management of VOD associated with GO.

PUBLICATIONS

Published Abstracts

PG096 LATE OCCURRENCE OF HEPATIC VENO-OCCCLUSIVE DISEASE FOLLOWING GEMTUZUMAB OZOGAMICIN: SUCCESSFUL TREATMENT WITH DEFIBROTIDE
Dipartimento di Oncologia ed Ematologia, Sezione di Ematologia, Università di Modena e Reggio Emilia, Modena, Italy

PU097 ONSET AND FOLLOW-UP: MOLECULAR ASPECTS AND ANTIGEN PROFILE IN ACUTE PROMYELOCYTIC LEUKEMIAS
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Over 95% of acute promyelocytic leukemias (APL) is characterized by a remarkable clinical response to all-trans retinoic acid (ATRA) and by the t(15;17) (q22;q12-23) translocation between the retinoic acid receptor α gene (RARα) on chromosome 17 and the PML gene on chromosome 15. This translocation results in the production of an aberrant transcript in which sequences derived from the 5' end of the PML gene are fused head-to-tail to the second intron of the RARα gene. Although precise molecular mechanisms remain largely unknown, clinical and experimental evidence point to a major role for the PML/RARα fusion protein in both maintenance of transformed phenotype of leukemic blasts and their clinical response to ATRA. Diagnosis of APL mainly on clinical presentation, cytomorphology and cytochemical analysis, however, molecular characterization of t(15;17) translocation is gaining increasing importance both because of the existence of ATRA-resistant molecular APL variants and the usefulness of molecular markers in the monitoring of minimal residual disease. Although combined treatment with ATRA and conventional chemotherapy achieves a complete clinical remission in over 97% of cases, almost 30% of these will experience a relapse during follow-up. Since it is generally found that the clinical relapse is invariably preceded by a molecular one, the routine use of a molecular marker provides the clinician with a very sensitive and specific tool for the early identification of patients at risk of relapse. The present paper reports on the usefulness of an RT-PCR based approach for the molecular characterization and follow-up of a group of 10 APL patients treated in our Institution between 1999 and 2002; of whom two experience molecular relapses.
Background and objectives: Adequate mobilization of PBSC may depend on the chemotherapy regimen used. The optimum PBSC mobilizing regimen in AML patients is still to be defined, particularly for those identified as hard to mobilize. Fludarabine-based regimens may severely impair stem cells mobilization and collection in leukemic patients. We investigated the mobilizing effect of a consolidation cycle with MINI-ICE in five leukemic patients (pts) all but one non responders to induction therapy and treated with intensive fludarabine-containing regimens. Design and methods: Five AML pts (4 females and 1 male) with median age of 34 years (range 29-63) entered the study. The diagnosis of acute leukemia according to the FAB classification was M4 in 2 pts (one with normal karyotype, one showing 47, XX,+22, inv 16), M0 in 1, not specified in the others. At the time of PBSC collection all patients were in complete remission (4 in I CR, 1 in II CR). One patient alone achieved complete remission after conventional DCE induction treatment (daunorubicin 50 mg/m²/day, days 1,3,5; cytosine arabinoside, Ara-C, 100 mg/m²/day continuous perfusion, days 1-5; etoposide 100 mg/m²/day, days 1-5) and received a second course as consolidation. Three pts resistant to DCE and another pt relapsed after ICE regimen (idarubicin 20 mg/m²/day days 1,3 and 5 orally), associated with G-CSF, 5 µg/kg/day, days 1-5 and 2 in the remaining, on median day +13 and +18 from the end of treatment, respectively, when CD34+ cell count was >10/mL. The median CD34+ count was 53.3/mL (range 15.1-112) in pts who received one apheresis versus 16.4/mL (range 13.5-17.5) in who received 2 aphereses. The median number of CD34+ cells/kg collected after 1 apheresis was 10.1 (range 1.8-15.2) versus 3.09 CD34+ cells/kg collected after 2 aphereses. Conclusions: Our results suggest that in AML patients heavily treated with fludarabine-based regimens, MINI-ICE may represent an effective mobilizing regimen with scanty extrahematological toxicity.

Funding: Supported in part by AIL Pesaro-Onlus.

Background and Objectives. Transfusional iron overload is a frequent finding in long-surviving acute leukemia patients. Some authors have investigated patients with hematologic malignancies for hereditary hemochromatosis (HH), but they have failed to demonstrate an association between high ferritin values and the presence of the most frequent HH gene mutations (C282Y and H63D). We analyzed the influence of HH gene mutations on iron status before and after treatment in a homogeneous group of long-surviving patients with acute myeloid leukemia (AML).

Design and Methods. We evaluated serum ferritin and transferrin saturation values and screened 12 HH gene mutations (C282Y and H63D). We analyzed the influence of HH gene mutations on iron status before and after treatment in a homogeneous group of long-surviving patients with acute myeloid leukemia (AML). The analysis of iron status was carried out at diagnosis, at the end of chemotherapy and during the follow-up. Results. High serum ferritin levels were associated with abnormal inflammatory parameters in 29/45 patients (64.4%) at diagnosis, in 10/45 patients (22.2%) at the end of induction chemotherapy and in none of the patients at the end of the follow-up. Initial mean ±SD serum ferritin level and transferrin saturation were 843.6±872.5 µg/L and 44.1±20.4% respectively. At the end of treatment mean ferritin level was 1539.9±780.8 µg/L and transferrin saturation was 51.0±15.3%. At the end of follow-up ferritin level was 1728±1038 µg/L and transferrin saturation 51.8±19.9%. In 24 patients (53.3%) ferritin values and/or transferrin saturation increased during the fol-
OUTCOME OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA OR HIGH RISK MYELODISPLASIA TREATED WITH AN ATTENUATED FLAIG REGIMEN


S.C. Ematologia, Dipartimento di Oncologia, Ospedale San Giovanni Battista, Torino; S.C. Ematologia, Ospedale SS. Arriago e Biagio, Alessandria; Divisone di Medicina B, Ospedale degli Infermi, Biella, Italy

Fifty three consecutive elderly patients with acute myeloid leukemia (AML = 35) or high risk myelodisplasia (MDS = 18) were treated with an attenuated FLAIG protocol. The median age was 67 years (range 60-76). The male/female ratio was 1.2. Successful karyotypic was obtained in 42 patients (79.2%): a normal karyotype was found in 27 patients and an isolated -Y in one patient. In 14 cases an unfavourable karyotype was recognized. The induction therapy was Fludarabine 30 mg/m2 (25 mg/m2 in patients > 70 yrs.) and Cytarabine 1 g/m2 for five days (4 days in patients > 70 yrs) plus Idarubicine 10 mg/m2 in 24 hours continuous perfusion on days 1-3-5. Glycosilated G-CSF was administered from day 6 to granulocyte recovery. Thirty six patients (67.9%) obtained a complete remission (CR) (AML 24 = 68.6% and MDS 12 = 66.7%, p=0.6). Six patients (3 AML, 3 MDS = 11.3%) died during the induction phase and 11 patients (8 AML, 3 MDS = 20.8%) were resistant. Patients in CR were treated with a first outpatient consolidation course with Fludarabine 30 mg/m2 (25 mg/m2 in patients > 70 yrs.), Cytarabine 1 g/m2 for 2 days and Idarubicine 10 mg/m2 on day 2. A second consolidation was given with Cytarabine 100 mg/m2/day s.c., Thioquanine 50 mg/m2/day for 5 days and oral Idarubicine 15 mg/m2/day on day 1. Maintenance treatment with Cytarabine 100 mg/m2 once a week and Thioquanine 50 mg/m2/day for 5 days a week was planned for one year or till relapse. Two patients with an HLA identical sibling underwent non-myeloablative allogeneic stem cell transplantation. The median survival (OS) was 10.8 months, with a median follow-up of 7 months (range 1.5-42) for censored patients and with an actuarial probability of survival at 42 months of 13%. The median disease-free survival (DFS) was 6.3 months. Age was the most important determinant of survival (median OS 12.9 months for patients < 67 years and 4.6 months for patients > 67 years; p=0.02) and remission duration (median DFS 10.5 months for patients < 67 years and 5.0 months for patients > 67 years; p=0.002). No difference in OS and DFS was observed between patients with AML or MDS and between patients with different cytogenetic risk. This attenuated FLAIG regimen provided an high incidence of CR with reduced toxicity and induction mortality. However the remission duration was short, particularly in the more advanced age group. The probability of long term survival was poor. Better consolidation and maintenance strategies should be devised to improve the outcome without affecting the quality of life.
isotypic control signal. Ionotropic and chronotropic activities were tested on guinea pig isolated atria preparations, and vasodilator activity was tested on guinea pig aortic strip preparations (see table below).

<table>
<thead>
<tr>
<th>Negative ionotropy</th>
<th>Cardiovascular activity</th>
<th>Negative chronotropy</th>
<th>Vasorelaxant activity</th>
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<tr>
<td>i.a.%</td>
<td>ECo (mM) (+ S.E.M.)</td>
<td>95% c.l.)</td>
<td>i.a.%</td>
</tr>
<tr>
<td>VRP</td>
<td>84 (2.1)</td>
<td>0.63 (0.40-0.80)</td>
<td>94 (3.4)</td>
</tr>
<tr>
<td>MN 36</td>
<td>55 (0.2)</td>
<td>1.11 (0.85-1.45)</td>
<td>65 (4.3)</td>
</tr>
<tr>
<td>CTS 4</td>
<td>34 (1.1)</td>
<td>—</td>
<td>76 (3.3)</td>
</tr>
<tr>
<td>CTS 9</td>
<td>69 (3.3)</td>
<td>0.55 (0.42-0.63)</td>
<td>71 (0.7)</td>
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<tr>
<td>CTS 27</td>
<td>46 (0.2)</td>
<td>—</td>
<td>66 (3.7)</td>
</tr>
<tr>
<td>CTS 41</td>
<td>46 (0.2)</td>
<td>—</td>
<td>67 (3.2)</td>
</tr>
</tbody>
</table>

Comparison between survival plots and relative ID50, obtained from K-562 cells treated with IDA, in presence or absence of inhibitor, showed a good activity of these compounds. All the resistance modifying agents induced a significant reduction (p< 0.01) of ID50 values in comparison to verapamil at each concentration but MM 36, CTS 27 and 41 demonstrated a strong activity. Results obtained from MNCs were superimposable in comparison to K-562. Further studies on apoptosis induction and pump functional analysis confirmed cytotoxic test results: M M 36, CTS 27 and 41 showed a striking activity in inhibiting Pgp efflux in K-562 and MNCs.

**PU103**

**RESVERATROL AFFECTS PROLIFERATION, APOPTOSIS AND DIFFERENTIATION OF ACUTE MYELOID LEUKEMIA BLASTS THROUGH THE INHIBITION OF NF-KB**

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The effects of STI571 on chronic myeloid leukemia demonstrate that the specific inhibition of the causal oncogene is the more promising approach to cure cancer. If in Bcr-Abl positive CML the molecular target is well defined, in the other cases of leukemias, and in particular acute myeloid leukemia, molecular approaches need to be found. Recent evidences attribute to the transcription factor NF-kB an essential role in acute myeloid leukemogenesis. Guzman et colleagues have demonstrated that NF-kB is costitutively activated in primitive acute Myeloid Leukemia cells and that the proteasome inhibitor MG-132, which affects NF-kB signaling, induces apoptosis of leukemic blasts. MG-132 treatment does not affect normal hemopoietic stem cells. Different NF-kB inhibitors have been described, with variable efficacy and toxicity. A potent and not toxic NF-kB inhibitor is Resveratrol, which is extracted from grape. The aim of this study was to evaluate the effects of Resveratrol in acute myeloid leukemia blast cells. We have studied 7 bone marrow samples of AML patients collected at diagnosis and the leukemic cell line HL-60. After separation on a ficoll density gradient bone marrow samples have been incubated with 90 µM of Resveratrol or with equal amount of solvent (control sample) for 4 days. After incubation the leukemic growth was strongly inhibited, with an associated induction of apoptosis, respect to the control sample. Moreover flow cytometry analysis has detected an increased expression of CD11b (in the leukemic cell line HL60), suggesting that Resveratrol not only inhibits proliferation but also induces differentiation of leukemic blasts. Resveratrol does not affect the number and the state of activation of normal lymphocytes. To investigate whether the described effects are mediated by NF-kB inhibition, we have used a sensitive ELISA method to detect the levels of NF-kB binding activity in treated and untreated blast cells. We have found that after Resveratrol treatment the NF-kB binding activity was reduced to the 20% of the basal level. These data candidate Resveratrol as a new drug in the treatment of acute myeloid leukemia patients.
patients ageing < 65 years with relapsed or refractory non M3-AML. Since march 98 to date we enrolled 38 patients: 18 patients presented a refractory AML, 15 an early first relapse (first CR duration < 1 year), and 5 a late first relapse (first CR > 1 year). At the time of salvage treatment median age was of 47,5 years (range 16-62); cytogenetic was favourable in 7 cases, intermediate in 22 cases and unfavourable in 9 cases. All patients received as salvage therapy: fludarabine 15 mg/m²/12h×5 d, ara-C 1 g/m²/12h × 5 d, idarubicine (Ida) 12 mg/m² × 2 d, ATRA 45 mg/m² × 10 d, G-CSF 300 µg from day -1 until day 5 and from day 12 to PMN recovery. Patients achieving CR or PR after the first cycle received a second cycle equal to the first one. Twenty five out of the 38 achieved a CR (65,8%), 22 after the first cycle and 3 after the second cycle. The CR rate in patients presenting with a refractory AML was 72% while 60% were the CR obtained in patients who relapsed. CRs according to the cytogenetic prognostic group were: 6/7 in the favourable group, 15/21 in the intermediate group, 3/10 in the unfavourable group. Ten patients showed a resistant disease to the salvage therapy, 3 patients died (9%) for infection during the aplastic phase. Median time to PMN>500/µL was 15 d (range 10-33) and PLT>20000/µL 16 d (range 7-66). During the aplastic phase we observed: 22 sepsis, 14 sustained by Gram+ and 8 by Gram-; 14 cases of pneumonia, microbiologically documented in 8 cases; 1 case of cellulitis and 1 pulmonary-cerebellar mucormicosis. Extra-hematologic toxicity of grade III or IV according to WHO was gastrointestinal in 8 cases, cardiac in two and hepatic in 1 case. Among the 25 patients in CR after the first cycle we observed: 22 sepsis, 14 sustained by Gram+ and 8 by Gram-; 14 cases of pneumonia, microbiologically documented in 8 cases; 1 case of cellulitis and 1 pulmonary-cerebellar mucormicosis. Extra-hematologic toxicity of grade III or IV according to WHO was gastrointestinal in 8 cases, cardiac in two and hepatic in 1 case. Among the 25 patients in CR after the first cycle we observed: 22 sepsis, 14 sustained by Gram+ and 8 by Gram-; 14 cases of pneumonia, microbiologically documented in 8 cases; 1 case of cellulitis and 1 pulmonary-cerebellar mucormicosis. Extra-hematologic toxicity of grade III or IV according to WHO was gastrointestinal in 8 cases, cardiac in two and hepatic in 1 case.

We describe the clinico-pathological features at diagnosis and follow-up of 12 cases of myeloid sarcoma (MS) observed at our institution; all had been initially misdiagnosed with malignant lymphoma (ML). Eight patients were males; median age at diagnosis was 45 years (range 4-84). In 9 patients, the diagnosis of MS was concomitant with a myelodysplastic phase (n=7) or acute myeloid leukemia (AML) (n=2). A four-year old patient, treated in the myelodysplastic phase, died during induction treatment, with no evidence of overt leukemia; in the remaining 2 patients the disease remained isolated for a long period of time. The primary sites of MS were epidural zone of the lumbar tract (n=3), skin (n=2), lymph node (n=2), mediastinum (n=2), pancreas (n=1), parotid gland (n=1), and uterus (n=1). In all patients, the diagnosis of MS was determined by immunohistochemical staining for CD45, CD43, CD68/KP1, CD68/PG-M1, CD34, MPO, and lysozyme. A median of 2.9 months (range 1-6) elapsed between the diagnosis of ML and that of MS, whereas a median of 5 months (range 2-44) elapsed between diagnosis of MS and that of AML (11 patients). Ten patients (9 with systemic disease and 1 with isolated MS) received intensive AML-like chemotherapy; 2 of them had previously been treated for ML. The two patients with isolated skin localization received low-dose chemotherapy following local radiotherapy, but both subsequently developed AML, at 38 and 44 months respectively. All patients achieved CR from MS, but only 4 patients (44%) achieved bone marrow CR. Median survival from MS diagnosis was 7 months (range 1-49), and at present time only 1 patient is still alive, 49 months after HLA-compatible donor stem cell transplantation. Our data stress on the importance of promptly and correctly diagnosing MS and of starting intensive treatment as soon as possible, for this subtype of patients who appear to have a very poor prognosis.
AM L is the most common type of acute leukemia usually arising between 55 and 65 years of age. Standard anthracycline and cytarabine-based regimens ensure a high percentage of complete remission (CR). However, the majority of patients eventually relapse or never achieve complete clearance of leukemic blasts thus determining a dismal prognosis. Old age and antecedent hematologic disorders, like myelodysplastic syndromes, represent negative prognostic factors for the achievement as well as for the maintenance of complete remission. The introduction of fludarabine-based regimens, namely FLANG or FLAG, has showed encouraging results even in this subset of patients. Since April 1998 we treated 24 patients affected by high risk AML with FLANG regimen, constituted by fludarabine, 25 mg/m²/daily from day 1 to 5, cytarabine, 500 mg/m²/daily from day 1 to 5, mitoxantrone, 6 mg/m²/daily from day 1 to 3, and filgrastim (G-CSF) 300 µg/daily from day 6 until ANC > 1x10⁹/L. There were 13 male and 11 female.

Seventeen patients has de novo AML, six had MDS-related AML and one patient suffered from a myeloid blastic crisis evolving from CML. Disease status at the start of treatment was the following: 13 patients at disease onset, 6 in first relapse and 4 with refractory disease. CR was achieved in 13 patients (54%) and PR was achieved in 3 (12.5%), for an overall response rate of 66.5%. CR rate was higher, although not statistically significant, in those patients treated at disease onset (61.5%) than in those with refractory or relapsed disease (25% and 50% respectively). Mean DFS and OS were 7.6 and 9.3 months, with longer overall survival in patients with newly diagnosed leukemia than relapsed or refractory (11.5 vs 8.2 and 5 months). Karyotype state was found to be inversely related to the prognosis: patients with an abnormal karyotype had a better long-term outcome than those with no chromosomal aberrations (OS 12.7 vs 7.6 months). Most of the patients experienced neutropenic fever but therapy was globally well tolerated, with two treatment-related deaths. These data show therefore that FLANG regimen is feasible in advanced age patients and in those with MDS-related AML and it allows to obtain complete remission in a significant proportion of patients. Furthermore it is also effective in patients with relapsed AML, even if previously treated with high dose cytarabine, as all our relapsed patients had.

The response to intensive induction in elderly patients with AML is usually disappointing since 1) age per se represents a poor prognostic factor, 2) the occurrence of cytogenetic abnormalities, though rare, may play an unfavourable role. Between February 1998 and May 2003, at our Institution, in 37 consecutive elderly pts (16 females and 21 males, median age 72 y, range 61-85y), conventional G-banding karyotypic analysis was carried-out at diagnosis. Results, out of 37 pts 25 (54.8%) exhibiting clonal (> 3 metaphases) abnormalities, 8(37.1%) resulted to have a normal karyotype, finally in 4 cases cytogenetics failed because of no metaphases: cytogenetic results are detailed in the table below.

<table>
<thead>
<tr>
<th>Cytogenetic Abnormality</th>
<th>n° patients</th>
<th>Frequency</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>+8</td>
<td>6</td>
<td>16.2%</td>
<td>70 m.a.*</td>
</tr>
<tr>
<td>t(9;11)(q22;q23)</td>
<td>4</td>
<td>10.8%</td>
<td>75*</td>
</tr>
<tr>
<td>Complex karyotypes</td>
<td>4</td>
<td>10.8%</td>
<td>69*</td>
</tr>
<tr>
<td>t(8;21)(q22;q22)</td>
<td>1</td>
<td>2,7%</td>
<td>65</td>
</tr>
<tr>
<td>-5/del(5q)</td>
<td>3</td>
<td>8,1%</td>
<td>72*</td>
</tr>
<tr>
<td>+13/del(13q)</td>
<td>1</td>
<td>2.7%</td>
<td>68</td>
</tr>
<tr>
<td>t(15;17)(q22;q22)</td>
<td>1</td>
<td>2.7%</td>
<td>66</td>
</tr>
<tr>
<td>t(8;11)(q22;q23)</td>
<td>1</td>
<td>2.7%</td>
<td>65</td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>8</td>
<td>21.7%</td>
<td>69 (m.a.)</td>
</tr>
<tr>
<td>No metaphases</td>
<td>4</td>
<td>10.8%</td>
<td>68 (m.a.)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>37</td>
<td>100%</td>
<td>72 (m.a.)</td>
</tr>
</tbody>
</table>

*m.a.: median age.

As to induction, 18 pts were considered eligible for an intensive treatment: such as DNR+ARA-C(3+7) schedule (12), Gimema LAM 99 (4), and FLA-G regimen (2), respectively. The remaining 21 cases, which median age was 78, were treated with palliative (11), or supportive(8) therapy, finally 2 patient died before treatment onset. As to response treatment 9 (50%) out the 18 patients treated with intensive regimen achieved CR, 1 PR; thus overall response rate (CR+PR) was 55.5%; 5 were refractory, and 3 died during induction. In all CR cases cytogenetics at CR time was found to be normal. None of the patients treated with palliative approach achieved response and died. Complex karyotype abnormalities are usually associated with poor treatment response, while in our series 3/4 (75%) achieved CR, and
only 4 of the 8 (50%) with normal cytogenetics were CRs. The high incidence (10.8%) of chromosome 13 abnormalities in our series does seem to contrast with findings in other experiences, referring an incidence of 1%; this event may be related to the increased median age (75 y) and male sex prevalence. In elderly AML a correct assessment of cytogenetics at diagnosis and during treatment phases should be considered mandatory, since it can allow to detect abnormalities, up to now, defined rare as well as to define their role in influencing treatment response and disease outcome.

PU107
COMPLETE CYTOGENETIC REMISSION IN ACUTE MYELOID LEUKEMIA WITH GRANULOCYTE-COLONY STIMULATING FACTOR WITHOUT CHEMOTHERAPY. A CASE REPORT
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In recent years, occasional complete remissions (CR) have been described with G-CSF alone in acute myeloid leukemia (AML). Moreover, G-CSF has been proposed as an alternative to donor lymphocyte infusion (DLI) in patients relapsed after allogeneic bone marrow transplantation (BMT). We describe an AML patient, carrying the t(9;11)(p22q23), who obtained a cytogenetic CR after treatment with G-CSF alone. A 50 years-old woman was diagnosed with AML M4 subtype. Peripheral blood counts were: WBC 1.2 × 10^9/L (PMN 15%, Ly 85%), PLT 95 × 10^9/L, Hb 8.1 g/dL. Bone marrow was hypercellular with 60% of blast cells. The karyotype was: 47 XX, t(9;11)(p22;q23), +8. Neither internal tandem duplication nor point mutation of FLT3 were present. Since a perianal abscess was present, it was decided to pre-treat the patient with G-CSF in an attempt to resolve the abscess before induction chemotherapy. A recombinant human G-CSF (lenograstim) was given at a dose of 263 mg/day, together with antibiotics. After 14 days of treatment peripheral blood and bone marrow aspirate were normal, and the t(9;11), +8 clone was no longer detectable. G-CSF treatment was discontinued, and the patient was observed without further treatment until she relapsed 8 months later. Relapse was hematologic and non-hematologic (breast). The morphology of leukemic blast cells and the karyotype were the same as at presentation. G-CSF treatment re-instituted, at the same dose for 5 weeks, but failed to re-induce remission. The remission was then obtained with a combination of high dose arabinosyl cytosine, fludarabine, idarubicine and etoposide. Until now other 15 case of AML in which a CR was obtained with G-CSF alone have been described. Many possible mechanisms of action have been proposed, including induction of maturation and apoptosis. A specific mechanism has been described for t(8;21)-positive AML, cases in which G-CSF induces direct blasts differentiation. In conclusion, G-CSF may be useful in selected AMI patients, not candidate to conventional treatments, especially in the elderly, with normohypocellular marrow, and low WBC-counts.

Funding: supported in part by AIL (Bologna and Pesaro), AIRC, COFIN 2001 (S. Tura) and COFIN 2002 (M. Baccarani) grants, Ateneo 60% (Baccarani and Piccaluga), Fondazione del Monte di Bologna and Ravenna.

PU108
EFFICACY AND FEASIBILITY OF GEMTUZUMAB OZOGAMICIN PLUS CYTARABINE IN THE TREATMENT OF POOR RISK ACUTE MYELOID LEUKEMIA
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Antibody-targeted chemotherapy is a promising approach in patients with hematologic malignancies. In particular, gemtuzumab ozogamicin (GO, formerly CMA-676), an anti-CD33 antibody linked to calicheamycin, was approved for the treatment of elderly patients with acute myeloid leukemia (AML) in relapse. In this setting, the reported overall response rate (CR + CRp) was 28%, with acceptable toxicity and with median survival of nearly 5 months for all patients and 11 months for patients showing a complete response. Only few data are available as regards the combination of GO with other chemotherapeutic agents. We treated with gemtuzumab ozogamicin (GO) based regimens thirty-four adult AML patients. GO was administered as single drug (9 mg/m², for a total of 2 doses every 14-28 days) in twenty-two cases of relapsed AML (sixteen of them were older than 60 years) (Group 1); GO in combination with cytarabine (GO 6 mg/m² day 1 and 4 mg/m² day 8; cytarabine 100 mg/m²/day, continuous intra-venous infusion, days 1-7) in nine patients (five with untreated AML aged over 60 years and four with relapsed/refractory AML (Group 2); GO (6 mg/m², day
In a retrospective analysis of our patients with acute myeloid leukemia undergoing induction chemotherapy, including cytarabine and anthracyclines, 3 patients developed typhlitis. They presented severe neutropenia, fever, abdominal pain and tenderness within 21 days from starting chemotherapy (median 14 days; range 8-21). Two patients underwent surgery (one right hemicolectomy and one resection of the ileum): they died after a week. The clinical course of the third patient was longer (3 months of abdominal pain and diarrhoea), he died without surgery and necropsy revealed ischaemic caecal necrosis with perforation and peritonitis, acute complete twisting of several loops of small bowel about their mesenteric basis of attachment, producing intestinal infarction. It is well known that surgical and/or medical approaches do not modify the evolution of typhlitis. In other reports the early recognition of typhlitis, the rapid recovery of the neutrophil count and the remission of the disease are the most important determinants of the results. In our experience the onset of the disease can be both acute, starting few days after the induction course and rapidly ending in the abdominal catastrophe, and chronic, slowly improving: in this case there is not a strict correspondence with neutropenia. The management of this life-threatening condition is still controversial: each case of typhlitis should be considered as a multifactorial process and each patient should be carefully evaluated between surgeon and hematologist.

Karyotype is the most important prognostic factor in AML. We evaluated the impact of cytogenetic on clinical outcome in 60 AML patients (pts) transplanted in first CR in our Unit between January 1995 and December 2001. The type of transplant was chosen according to availability of an HLA-matched sibling donor. Pts characteristics at diagnosis: median age 43 years, M/F 22/38, median WBC count 16×10⁹/L, median LDH 700 UI/L, de novo AML 47 and s-AML 13. Cytogenetic abnormalities, grouped according to MRC criteria, were classified as favourable in 9 cases, intermediate in 37 and adverse in 14. The majority of pts achieved CR after the first cycle of induction (49 pts vs 11 pts), median time form CR to transplant was 4 months. 39 pts were submitted to autologous BMT (29 from bone marrow and 10 from PB); the conditioning regimen was BUCY. 21 pts underwent to allogeneic transplant (12 from bone marrow and 9 from PB); the conditioning regimens were TTCTY (13 pts) or BUCY (8 pts). Results. at November 2002 the median follow-up was 46 months. The 5 years projected survival was 69% with no significant difference between autologous and allogeneic setting. The TRM was 2% in autologous (1 DVT and 10 from PB); the conditioning regimen was BUCY. 21 pts underwent to allogeneic transplant (12 from bone marrow and 9 from PB); the conditioning regimens were TTCTY (13 pts) or BUCY (8 pts). Results. at November 2002 the median follow-up was 46 months. The 5 years projected survival was 69% with no significant difference between autologous and allogeneic setting. The TRM was 2% in autologous (1 DVT and 24% in allogeneic (4 aGVHD and 1 encephalitis). LFS at 5 years was 68%, 64% for autologous and 76% for allogeneic. By univariate and multivariate analysis performed for age, sex, WBC count, LDH, type of leukemia
(de novo vs s-AML) and karyotype groups, diagnostic cytogenetics was the most powerful prognostic factor on LFS (p=0.03). The risk of relapse was about 2 times higher between the three prognostic groups (HR 2.28; CI 95%: 1.05-5.02). The LFS was 80% for autologous and 100% for allogeneic transplant within the favorable karyotype group while was 40% for autologous and 50% for allogeneic in the adverse and 65% vs 100% in the intermediate group, showing a consistent difference within the last group. Conclusions. Cytogenetics remains the most important prognostic factor for patients affected by AML treated with transplantation and the allogeneic transplant was confirmed as the more effective procedure to prevent relapse especially in patients with intermediate risk karyotype.

**PU111**

**MAINTENANCE THERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA IMPROVES SURVIVAL**

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The outcome of elderly patients with acute myeloid leukemia following remission-induction therapy is still disappointing. Decreased efficacy, low tolerance to therapy with frequent side effects and age-related comitant diseases limit treatment in this particular patient group. The efficacy of postremission maintenance therapy is still being investigated. Recently, the EORTC and the Dutch-Belgian Hemato-Oncology Cooperative Group have demonstrated an advantage in maintenance therapy with low dose Ara-C in terms of disease-free survival (DFS) as compared to no therapy following remission, though no benefit in overall survival (OS) has been obtained. We evaluated the effects of postremission therapy with Ara-C associated with thioguanine on DFS and on OS and its tolerability in a group of patients with AML over 60 years of age. Seventeen consecutive patients obtaining first complete remission were included in the study to receive Ara-C 100 mg/m² bid + Thioguanine 100 mg/m² bid for 5 days at 30-day intervals from complete remission up to relapse. Median age was 70 (range 62-84) years. Twelve patients had concomitant diseases (6 cardiovascular, 3 endocrine, 1 solid tumors, 1 chronic respiratory disease, 1 chronic renal failure). Two patients had AML following MDS and one following MF. One third of patients had a poor performance status (WHO = 2). A median of 6 cycles (range 1-25) were completed with good tolerability. Only one patient developed Grade 4 hematologic toxicity with severe leukopenia (≤500) and thrombocytopenia (≤10,000) which lasted 4 days. Seven patients (41%) did not require transfusions; of the remaining 10 patients, 1 required a total of 7 transfusions in 3 months and 9 required from 1 to 3 transfusions during the period of therapy. Less than half of the patients required platelet infusions, up to a maximum of 3 infusions during the entire period of observation. Therapeutic cycles did not require prolonged hospitalizations beyond the expected time span for administration (5 days). Seven patients (41.2%) are alive at 65.5 months from remission with a median OS of 21.0 (range 3.0-65.5) months. Median DFS is 12.6 months with 6 patients (35.3%) still in complete remission. These results suggest that maintenance therapy with standard dose Ara-C associated with thioguanine may give a survival advantage in terms of DFS and, most importantly, of OS in elderly patients with AML.

**PU112**

**TWO CASES OF CD7+ AND CD56+ MYELOID/NATURAL KILLER CELL PRECURSOR ACUTE LEUKEMIA: A DISTINCT HEMATOLOGY DISEASE WITH PARTICULAR EXTRAMEDULLARY INVOLVEMENT**

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Malignancies of natural killer (NK) cells have increasingly been recognized as a distinct biologic and clinical disease. In this report we describe two cases of CD7+ and CD56+ myeloid and NK Cell Precursor acute leukemia: the first patient was a 16-years-old girl previously treated with chemotherapy (CEVAIE schedule) and local radiation (44.8 Gy) for an embrional rhabdomyosarcoma localized on left arm (TIA;IRS III); the second patient was 62 years old man at diagnosis. On admission, both of them showed severe anemia (Hb 6.2 and 7.1 g/dL), thrombocytopenia (PLT 45,000 and 54,000/mm³), leukopenia (WBC 3.000 and 3.800/mm³) with only 10% of myeloid phenotype blasts cells on peripheral blood smears. Most important, in both patients a prominent extramedullary involvement was evident as initial presentation, with diffuse skin lesions whose histological analysis revealed a cell infiltration mainly located in the dermis. Actually, the histopathologic examination of tumor cells on biopsed specimens showed a lymphoblastic-like morphology, with large cell size, round to moderately irregular nuclei and prominent nucleoli, pale cytoplasm and a lack of azurophilic granules. The immunohistochemical analysis showed the expression of CD4 and LCA markers, while CD20, CD79a, CD3, CD8, MPO, CD13, CD57 and CD68 were negative. The bone marrow smears examined showed diffuse infiltration by monotonous proliferation of immature mononuclear cells, with finely distributed nuclear chro-
matin, prominent nucleoli and a large amount of faint basophilic cytoplasm without granulations. Cytoplasmic vacuolation was frequent. The immunophenotype of leukemic cells was characterized by the expression of CD13, CD56, CD4, CD7 and CD38 without MPO reactivity. Furthermore, cyogenetic study demonstrated the following complex karyotype: 45, XX, t(1;6)(q21; q24), add(7)(p22) in the young female, 46 XY, add(2)(p25), add(11)(p15) in the male patient. Most important, in both of them del(9) and del(13) have been detected. Patients received standard induction therapy for AML. The male patient died after 17 days from the beginning of the induction therapy when he started to present neurological symptoms and signs. Actually, central nervous system involvement has been demonstrated by the presence at lumbar puncture (LP) of pleocytosis and blast cells on cerebral spinal fluid. For this reason, the female patient underwent to CNS prophylaxis with intrathecal methotrexate (12 mg), ARA-C (40mg) and Prednisone (40 mg). Complete remission was obtained and the cyogenetic analysis revealed a normal karyotype (46, XX). After two months this patient underwent matched unrelated donor transplant and, to date, she is still alive in complete remission. Conclusions. CD7+ CD56+ myeloid/natural killer acute leukemia has been associated with a poor prognosis and a high incidence of neurologic symptoms, but it shows some response to intensive chemotherapy. In our opinion CNS effective prophylaxis should be used in the treatment of this uncommon type of acute myeloid leukemia.

PU113
FLAG REGIMEN IN POOR PROGNOSIS ACUTE LEUKEMIA.
A RETROSPECTIVE ANALYSIS ON 109 CASES

FLAG regimen (Fludarabine Ara-C, G-CSF) has been already proposed as an effective and well tolerated regimen for poor-prognosis acute leukemia patients. In this study we examined retrospectively the results of this treatment schedule in a consistent series of refractory/relapsed acute leukemia patients treated in 13 GIMEMA Centers between 1994 and 2003. 109 patients were registered in the study, 82 AM L and 27 ALL. Among the 82 AM L patients (49M/33F; median age 50 years, range 17-74), 38 were in relapse (31 Ist, 5 IInd, 2 III), 38 were resistant to first line and 6 were in resistant relapse. 21/31 patients treated in Ist relapse had a duration of Ist CR ≤ 12 months. Among the 27 ALL patients (19M/8F; median age 27 years, range 18-72), 18 were in relapse (14 1st, 4 2nd), 5 were refractory to first line and 4 were in resistant relapse. 93 patients had fever (median 4 days, range 1-30), with a documented aetiology in 47% of cases. Serious infective complications were 23/109 (20%), in particular 4 systemic fungal infections, 3 bacterial pneumonia, 2 fungal pneumonia, 10 pneumonia of unknown origin, 1 synusitis and 2 infections of skin and soft tissue. Extra-hematological (grade 3-4) toxicity was 11% overall, the incidence of mucositis was very low (6%), nausea and vomiting were reported in 3% of cases and alopecia was not reported. Median time for PMN reconstitution >0.5x10⁹/L and for PLTS >20x10⁹/L in patients achieving CR was 20 days (10-37) and 20 days (5-30) respectively, and complete remission was obtained in 50/109 cases (46%). In the AM L group, CR was achieved in 40/82 patients (37 after 1 cycle, 3 after 2 cycles), that means an overall CR rate of 49%; 33 patients were refractory (40%) and nine patients (10%) died during induction. Stratifying AML patients in relapse, refractory and resistant relapses evaluation of response showed a 53% of CR rate in the refractory group (20 pts), 45% in the relapse group (17 pts) while 3/6 pts (37.5%) achieved CR in the resistant relapse category. 13/40 patients achieving CR (32%) underwent a transplant procedure (9 autologous and 4 allogeneic). Overall survival probability projected at 12 months is 34%, with a median survival of 9 months. No statistically significant difference in survival was reported comparing the two groups of relapsed and refractory patients (p=0.7). Twelve-month projected disease-free survival of the whole population is 27% (median 7.5 months), and again no difference was detected comparing the relapsed and the refractory group. Fisher's exact test was employed to evaluate the impact of karyotypic abnormalities on response rate, showing no correlation between cytogenetic risk group (high risk vs intermediate risk) and response to induction. In the ALL group, overall CR rate was 37%; among the 10 patients achieving CR 8 belonged to the relapsed group and 2 to the refractory group. No patient in the resistant relapse group achieved CR. One patient (3%) died during induction. Seven out of 10 CR patients (70%) underwent a transplant procedure (2 autologous, 5 allogeneic). Overall survival of ALL patients is 16% projected at 12 months, with a median survival of 6.4 months. DFS projected at 7 months is 30%, with a median of 6.5 months. Our data confirm the antileukemic activity of FLAG regimen without the addition of anthracyclines especially in the setting of poor prognosis acute myeloid leukemia. In our series no difference in CR rate was reported according to cytogenetic findings, toxicity was very low, providing a bridge to a transplantation procedure in a high proportion (40%) of responsive patients.
A SIMPLE SCORING SYSTEM TO EVALUATE PROGNOSIS OF ELDERLY PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA (AML) NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY
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Acute myelogenous leukemia (AML) is a common disease in people aged > 60 years: more than 50% of these patients are not eligible for intensive chemotherapy and are managed with conservative approaches. To identify different prognostic groups, simple clinical parameters at onset (age, gender, peripheral blood counts, peripheral blasts, PS, previous myelodysplasia) were retrospectively evaluated in 212 consecutive patients (124 males and 88 females, median age 72 years, range 60-90) with AML diagnosed at our institution from 1/88 to 12/98 and not eligible for intensive chemotherapy. Older age (> 75 years), poor PS (> 2 according WHO), lower PLTS count (< 50 × 10^9/L) and higher absolute peripheral blast count (> 5.0 × 10^9/L) showed a poor prognostic significance on survival in multivariate analysis. On this basis, patients were divided by age in 2 groups: 143 patients aged < 75 years (group A) and 69 patients aged ≥ 75 years (group B). In both groups patients were then scored according to PS, PLTS count and peripheral blasts: score 0 (no risk factor), score 1 (1 risk factor), score 2 (2-3 risk factors). Results are shown in Table 1. In conclusion, this score system is very easy to apply, as it is based on simple clinical parameters evaluable also in general medicine departments, where these patients are often addressed; moreover, it seems capable of discriminating patients with a relatively more indolent course from patients with more aggressive disease. If reproducible in larger cohorts of elderly AML patients, it could help for better tailored treatment choices.

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<th>Score</th>
<th>N</th>
<th>MedSurv (days)</th>
<th>p</th>
<th>N</th>
<th>MedSurv (days)</th>
<th>p</th>
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<tbody>
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<td>69</td>
<td>182 (p&lt;0.001)</td>
<td>29</td>
<td>130 (p&lt;0.0001)</td>
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<tr>
<td>Score 1</td>
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<td>23</td>
<td>173 (p=0.0768)</td>
<td>20</td>
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PU115
ARSENIC TRIOXIDE IN THE TREATMENT OF ADVANCED ACUTE PROMYELOCYTIC LEUKEMIA (APL)

In order to evaluate the efficacy of Arsenic Trioxide (ATO) in patients with advanced APL, we treated 11 patients (6 male and 5 female, median age 38 years, range 5 - 62) in 1st resistant relapse (4 patients) or 2nd relapse (7 patients) with ATO (0.15 mg/kg daily for a maximum of 60 days) until hematologic complete remission (HCR). Three patients died from cerebral hemorrhage during an APL differentiation syndrome while 8 patients (73%) achieved hematologic complete remission (HCR) after a median treatment duration of 37.5 days (range 28 - 50) and a median cumulative dose of 300 mg (range 108-564). APL differentiation syndrome developed in 3 cases: extra-haematological toxicity included a Q-T prolongation in 2/11 patients (18%), which however did not require ATO discontinuation, and severe peripheral neuropathy in 2/11 (18%) cases. Hyperleukocytosis (WBC > 10 × 10^9/L) during the induction treatment with ATO was observed in 6 /11 patients (54.5%), with a median WBC count peak of 57 × 10^9/L: cytotoxic drugs were added in only one patient during induction. 7/8 patients received 1 cycle of ATO as consolidation treatment. Among the 8 patients in HCR, 6 became PCR-negative for PML/RARα after the first cycle of ATO and the remaining 2 entered molecular remission after consolidation. As to follow-up, 1 patient was lost in molecular CR after 2 months, 1 patient did not receive any other treatment and relapsed after 3 months, 2 patients received one further cycle of ATRA + Idarubicin and both relapsed after 3 and 4 months respectively. The remaining 4 patients underwent transplant procedures: 2 of them received an autologous bone marrow transplantation (BMT) and both relapsed after 13 and 22 months respectively, while 2 received an allogeneic BMT (1 died in molecular relapse after 20 months, 1 is still alive in molecular CR after 20 months). Our data suggest that ATO may be effective also in very advanced APL, but for the short CR durability this therapy seems indicated in patients eligible for transplant procedures.
LATE RELAPSE OF ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH ALL-TRANS RETINOIC ACID AND CHEMOTHERAPY: REPORT OF TWO CASES


Division of Hematology and Stem cell Transplantation Unit, AORN A. Cardarelli, Napoli, *Division of Hematology, Federico II University, Napoli, Italy

Acute promyelocytic leukemia (APL) shows peculiar morphologic, immunophenotypic, cytogenetic and molecular findings. During last decade, the prognosis of the disease is markedly improved since the introduction of combined treatment with all-trans retinoic acid (ATRA) plus chemotherapy. Currently, about 70% of patients achieve long-term complete remission (CR) and cure. Of the 20-30% of patients who relapse, recurrence occurs within 2-3 years from CR achievement, late relapses being extremely rare. Here we describe two APL patients, treated with the AIDA-0493 protocol, who relapsed at 9 and 7 years from CR achievement, respectively. Case #1. C.D., a 21-year-old male, was diagnosed with classical APL on June 1993. Immunophenotype, cytogenetics and molecular analysis (PM L/RAR α bcr1-2) were consistent with APL diagnosis, the patient achieved hematologic and molecular remission and discontinued treatment on November 1993. A bone marrow evaluation, done on February 1998, confirmed molecular remission. On November 2002, after a disease free survival (DFS) of 111 months, the patient presented with astenia, petechiae and fever. Blood count revealed pancytopenia, while bone marrow showed 90% promyelocytes with frequent Auer rods. Molecular analysis revealed identical bcr rearrangement as at diagnosis. The patient received chemotherapy with AIDA-2000 protocol and after the first consolidation obtained molecular remission. At the time of writing, he is alive in second hematologic and molecular remission, 8 months from relapse. Case #2. G.E., a 18-year-old male, was diagnosed as having classical APL on January 1994. Immunophenotype, cytogenetics and molecular analysis (PM L/RAR α bcr1-2) were consistent with a diagnosis of APL; the patient achieved hematologic and molecular remission and discontinued maintenance treatment with ATRA on May 1996. A bone marrow evaluation, done on January 1999, confirmed molecular remission. On February 2001, after a DFS of 84 months, the patient presented with pancytopenia, with bone marrow showing 100% promyelocytes infiltration. Molecular analysis revealed identical bcr rearrangement as at diagnosis. The patient received the current AIDA-2000 protocol, obtaining molecular remission after the second consolidation. At the time of writing, he is alive in second hematologic and molecular remission, 26 months after relapse. The occurrence of late relapse in APL is infrequent, rising the doubt of a second leukemogenic event. However, at relapse both our patients presented with the same immunophenotypic pattern and the same molecular lesion as at diagnosis, suggesting that in both cases the relapse was due to reemergence of the initial clone. Given the long period of DFS, both patients were treated with ATRA plus idarubicin, and both achieved a second molecular remission. Both patients are receiving maintenance therapy including ATRA; no transplantation procedure has been planned along with careful molecular monitoring. Of note, in the second patient a bone marrow harvest had been performed during the first molecular remission, and stored. We conclude that, although infrequently, patients with APL treated with modern combination therapy can experience very late relapse and can be rescued with identical treatment administered at diagnosis.

CLINICO-BIOLOGICAL FEATURES AND OUTCOME OF ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS WITH PERSISTENT PCR-DETECTABLE DISEASE AFTER THE AIDA FRONT-LINE INDUCTION AND CONSOLIDATION THERAPY


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In this study, we analysed the clinico-biological features at presentation of acute promyelocytic leukemia (APL) patients who showed PCR-detectable residual disease as compared to those of patients achieving molecular remission after the AIDA induction and consolidation. A total of 677 patients enrolled in the AIDA 0493 study during the period 1993-2000 completed induction and consolidation therapy. Of these, 23 (3.4%) tested PCR +ve and 654 PCR -ve at this time point. Compared to patients attaining molecular remission (n=654), patients who tested PCR +ve at the end of consolidation showed lower fibrinogen levels (p=0.05) whereas no statistically significant differences were observed as regarding median age, sex distribution, WBC and platelet counts, morphologic subtype (M 3 or M 3v) and PM L/RARα transcript type. The immunophenotypic profile showed no apparent
differences between post-consolidation PCR-ve and PCR-ve groups. Seven of the 23 patients received immediate salvage therapy while still in hematologic remission (HR) and 16 patients underwent hematologic relapse within a median time of 1 month (range 1-10) after confirmation of PCR-positivity and received salvage for overt disease recurrence. In the group of 7 patients treated in HR, 3 patients received salvage chemotherapy (CHT) followed by autologous SCT and are alive in HR and molecular remission (MR) at 64, 96 and 98 mos., while 4 patients underwent allogeneic SCT with no previous salvage CHT and are alive in hematologic and molecular remission at 64, 92, 98 and 118 mos. In the group of 16 patients treated for hematologic relapse, 9 patients received CHT alone and died of progressive disease; 5 patients were treated by CHT and allogeneic SCT, of these, 3 died of progressive disease and 2 are alive in HR and MR at 62 and 74 mos., respectively; finally, 2 patients received CHT followed by autologous SCT and both died for progressive disease. Disease progression was associated in this series with a high incidence of extramedullary leukemia infiltration. In fact, CNS involvement was documented in 8 patients while one patient had APL localisation in the external ear. Our findings indicate that APL patients molecularly resistant to the AIDA protocol have no distinguishing features at presentation. Outcome results suggest the need of early therapeutic intervention with aggressive treatment prior to the occurrence of hematologic relapse.

**PU118**

**INCIDENCE OF LATE RELAPSE (> 5 YEARS) IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA**


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Late relapse (5 - 20 years after diagnosis) have been already reported in many hematologic diseases, but have been seldom described up to now in patients with Acute Promyelocytic Leukemia (APL). We report 4 cases of APL patients in late relapse observed at our Institution in a 10-year period (1988 - 12/97). Clinical characteristics at 1st APL diagnosis are shown in the Table 1: the diagnosis of late relapse was made 60, 61, 71 and 155 months respectively after the onset of APL, and 31, 39, 52 and 121 months respectively after the stop of 1st treatment plan.

Table 1. Main characteristics of patients at time of APL diagnosis and treatments received.

<table>
<thead>
<tr>
<th>Case</th>
<th>Pt.</th>
<th>Sex/Age</th>
<th>WBC (x10⁹/l)</th>
<th>Platelets (x10⁹/l)</th>
<th>PML/RARA isoform</th>
<th>Front line Risk</th>
<th>Karyotype</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>M/22</td>
<td>5.4</td>
<td>15</td>
<td>LAP88</td>
<td>I</td>
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<td>30.3</td>
<td>9</td>
<td>LAP88</td>
<td>I</td>
<td>t(15;17)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>F/16</td>
<td>12</td>
<td>16.3</td>
<td>BCR1</td>
<td>AIDA H</td>
<td>G</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>M/18</td>
<td>3.5</td>
<td>18</td>
<td>BCR1</td>
<td>AIDA H</td>
<td>I</td>
</tr>
</tbody>
</table>

Patient #1 had a marrow relapse, patient #2 a marrow relapse with ear localization, patient #3 a molecular relapse with ear localization and patient #4 an isolated ear localization while in molecular complete remission (MCR); as a matter of fact, in 3/4 cases there was an ear localization of disease when the relapse occurred. Patient #1 was treated with ATRA alone, followed by allogeneic bone marrow transplantation: he is alive and in 2nd MCR after 72 months from transplantation. The other 3 patients received APL-relapse GIMEMA protocol (ATRA + Mitoxantrone + Cytarabine) and are all in 2nd MCR after 18, 20 and 30 months, respectively. These cases demonstrate that in APL patients late relapse is a less rare event than previously reported, with a good prognosis with reinduction standard treatments: the high rate of ear involvement at relapse in our cases might be explained considering the ear like a disease sanctuary, in which residual blast cells could escape from treatment and eventually proliferate again after a long period.

**PU119**

**FLAG-IDA IN THE TREATMENT OF REFRACTORY/RELAPSED ACUTE MYELOID LEUKEMIA: SINGLE CENTER EXPERIENCE**

Pastore D,* Specchia G,*° Carluccio P,* Liso A,* Mestice A,* Rizzi R,* Greco G,* Buquicchio C,* Ciuffreda L,* Guaragna GL,* Pietrantuono G,* Liso V,*

*Department of Hematology, University of Bari; °Hematology, University and Hospital of Foggia, Italy

Patients with relapsed or refractory acute myeloid leukemia (AML) have poor outcome and unfavourable response to chemotherapy; the morbidity of further chemotherapy is considerable, as most patients have already been exposed to intensive chemotherapy. The achievement of CR1 in refractory patients and CR2 in relapsed patients may offer the opportunity of further treatment, including transplantation procedures, provided that the toxicity of the salvage regimen is acceptable. We evaluated the efficacy and toxicity profiles of the combination of fludarabine, high dose cytosine-arabinoside, idarubicin and G-CSF in refractory/relapsed AM L patients. Between October 1998 and December 2002, 54 AM L patients were treated with FLAG-IDA (flu-
malities. In a group of 352 cases of adult acute leukemias in a large cohort of adult acute leukemia patients we evaluated the correlation between t(15;17), t(8;21) and inv(16) in AML and t(9;22) and some structural chromosomal abnormalities such as residual disease and to predict the presence of atypical phenotypes, serving to identify minimal residual disease in AML-M0 and AML-M7. Furthermore, the prediction of AML-M0 and AML-M7 is particularly important for the correct diagnosis and classification of acute lymphoblastic leukemia and is particularly important for the correct diagnosis and classification of acute lymphoblastic leukemia (130 B cell acute lymphoblastic leukemia, 222 acute myeloid leukemia) we analysed immunophenotypic findings with a large panel of monoclonal antibodies, and karyotypic abnormalities by conventional cytogenetic and/or RT-PCR. Out of 130 B-ALL cases, 30 (23%) had the t(9;22) and 5 (4%) the t(4;11). In the AML group (7 M0, 14 M1, 66 M2, 67 M3, 5 M3v, 28 M4, 6 M4eo, 21 M5, 6 M6 and 2 M7) the t(15;17) was found in all M3 cases, while the t(8;21) was observed in 9% (1 M0, 16 M2, 2 M4) and the inv(16) in 3% (1 M2, 5 M4eo). In ALL with t(9;22) we observed a significantly higher expression of CD10, CD34 and CD13 (p=0.006, 0.012, 0.034, respectively) while in AML with t(4;11) there was a high frequency of CD15 expression (p=0.001). The predictive value of immunophenotyping for the t(9;22) was even more evident (p<0.0001) when antigen coexpression (CD10/CD13/CD33/CD34) was investigated. In M3 cases we found a lower expression of HLA-DR and CD34 (p<0.001) and a higher expression of CD2 (v=0.02); all but one variant M3 (80%) were CD2+ vs. only 14 out of 67 (21%) classical M3. The t(8;21) cases demonstrated a significantly higher frequency of CD19 (p<0.001) and CD56, CD34, CD15, HLA-DR expression (p<0.05); in cases with inv(16) a high frequency of CD2 expression (p=0.004) was observed. Our results confirm the correlation reported between some structural chromosomal abnormalities and antigen expression; however, CD13 and CD15 expression in B-ALL does not seem to be confined to cases with t(9;22) and t(4;11), respectively. In AML cases, too, there seems to be a strong but not strict correlation between CD19 and CD2 expression with t(8;21) and inv(16), respectively, and the lack of HLA-DR, CD34 and CD15 expression in cases with t(15;17). Further studies in a large series are needed to investigate whether quantitative immunophenotyping could improve the identification of cytogenetically distinct subgroups of acute leukemias.

Myelodysplastic Syndromes

PU121

VERY LONG SURVIVAL IN A PATIENT WITH MYELODYSPLASTIC SYNDROME PRESENTING AN 11Q23 INTERSTITIAL DELETION, RESEMBLING A 5Q- SYNDROME


Hematology Unit, *Pathology Departement, Azienda Sanitaria Locale ASL Viterbo, Italy

We report the case of an old patient with myelodysplastic syndrome (MDS) presenting an interstitial deletion of the long arm of chromosome 11 (del 11q23), darabine 30 mg/m², ARA-C 2 g/m² for 5 days, idarubicin 10 mg/m² for 3 days and G-CSF 5 mcg/kg from day +6 until neutrophil recovery). Thirty-four patients were in remission after conventional chemotherapy including cytarabine, etoposide and daunorubicin or mitoxantrone according to GIMEMA protocols; 5 were in remission after autologous peripheral stem cells transplantation and 3 after allogeneic bone marrow transplantation. Twelve patients had refractory disease after induction chemotherapy including standard doses of cytarabine (10 days), mitoxantrone or daunorubicin (3 days) and etoposide (5 days). Recovery of neutrophils and platelets required a median of 19 and 22 days from the start of therapy. Complete remission (CR) was obtained in 28/54 (51.8%) patients and 4/54 (7.4%) died during reinduction therapy; 2 due to cerebral hemorrhage, 1 due to fungemia (C. tropicalis) and 1 due to multi-organ failure. Fever >38.5°C was observed in 50/54 (92.5%) patients, 34 being FUO and 16 documented infections; 46/54 (85.1%) developed mucositis and 22/54 (40.7%) had grade 2 WHO transient liver toxicity. After achieving CR 13 patients received allogeneic stem cell transplantation, 5 patients received autologous stem cell transplantation, 4 were judged unable to receive any further therapy and 6 refused other therapy. Twelve patients are at present in continuous CR after a median follow-up of 10 months (range 4-30). In our experience FLAG-IDA is a well tolerated and effective regimen in relapsed/refractory AML; the toxicity is acceptable, enabling most patients to receive further treatment, including transplantation procedures.

PU120

MULTIPARAMETRIC IMMUNOPHENOTYPING IN ADULT ACUTE LEUKEMIA: CORRELATION WITH KARYOTYPIC ABNORMALITIES


Department of Hematology, University of Bari, Italy

Immunophenotyping is an essential method for the diagnosis and classification of acute lymphoblastic leukemia and is particularly important for the correct identification of AM L-M0 and AM L-M7. Furthermore, immunophenotyping has been reported to be able to detect atypical phenotypes, serving to identify minimal residual disease and to predict the presence of some structural chromosomal abnormalities such as t(15;17), t(8;21) and inv(16) in AML and t(9;22) and t(4;11) in ALL. We evaluated the correlation between immunophenotypic findings and karyotypic abnormalities in a large cohort of adult acute leukemia patients in order to assess whether immunophenotyping can suggest the presence of structural chromosomal abnormalities. In a group of 352 cases of adult acute leukemia we report the case of an old patient with myelodysplastic syndrome (MDS) presenting an interstitial deletion of the long arm of chromosome 11 (del 11q23),...
showing morphological and clinical features resembling a 5q- syndrome, slowly progressive disease and long survival. We have followed an 83-year-old man since March 1995 because of mild macrocytic anemia and moderate thrombocytosis. His past medical history and the physical and instrumental examinations were not remarkable. The bone marrow (BM) was hypercellular and showed an increased number of nonlobulated mononuclear megakaryocytic cells, erythroid hyperplasia and no excess of blasts. Cytogenetic analysis revealed the interstitial deletion of long arm of chromosome 5, involving the q23 region. He was diagnosed as having MDS (refractory anemia). Two years later, when it was developed, according to International Prognostic Scoring System (IPSS), the patient was classified in the intermediate 1 risk group (score 0.5). He was monitored without being given any medication until 24 months later, when he required transfusion because of the asymptomatic anemia. To date, 98 months after the primary MDS diagnosis, the patient is 93-year-old and has anemia, requiring about one red blood cell unit every week, accompanied by a moderate thrombocytosis and a recently developed mild neutropenia. A recently performed re-evaluation showed a 7 percent of and no other remarkable changes on BM. The karyotype analysis revealed the presence of both a normal population (20%) and an 80% abnormal clone. Interphase cytogenetic analysis performed on two BM samples evaluating 10 (8 abnormal) and 14 (12 abnormal) metaphases respectively, excluded the critical genes on 5q. Some clinical and biological aspects of this case, such as the similar BM morphologic features, the transfusion dependent anemia, the thrombocytosis and the favourable course, resemble a 5q- syndrome. Karyotyping is important for the diagnosis and prognosis of MDS. The 11q23 abnormalities have been found in several hematologic disorders, including a minority of MDS, and are reported to be prognostically adverse. In our case, this abnormality was found together the typical features of a 5q- syndrome and is associated with a good outcome. Studies on large MDS series are required to establish whether this observation is sporadic or could be correlated to a distinctive clinical entity.

PU122
ANTI-TUMOR NECROSIS FACTOR THERAPY WITH A MONOCLONAL CHIMERIC ANTIBODY (INFlixIMAB) IN MYELODISPLASTIC SYNDROME. TWO CASE REPORTS
Capochiani E
Hematology Section, Oncology Department, Spedali Riuniti di Livorno, Livorno, Italy

The myelodysplastic syndromes (MDS) are a heterogeneous group of stem cell disorders characterized by pancytopenia, ineffective hematopoiesis and a risk of progression to aplastic anemia or acute myeloid leukemia. In the last few years some progress has been made in the understanding of the different disease mechanisms; laboratory and clinical observations have suggested that in some MDS, immune mechanisms may contribute to the impaired blood cell production. Tumour necrosis factor α (TNF-α) levels have been reported to be elevated in MDS and correlated with the number of apoptotic cells in the marrow, degree of anemia and prognosis. Infliximab (Remicade) is a chimaeric IgG-κ human and murine antibody that inhibits TNF-α, and the inhibition of TNF production can be a new therapeutic strategy in pancytopenic MDS. We have treated two patients, (male, age 62 and 67), with refractory anemia with ringed sideroblast (RARS) in according with WHO classification, and pancytopenia. Patient #1: Hb 6.7 g/dL, WBC 0.9×10^6/L µL, PLT 54×10^6/L µL; Patient #2: Hb 8.2 g/dL, WBC 1.4×10^6/L µL, PLT 43×10^6/L µL. IPSS score 0.5. Non chromosomal abnormalities were founded in bone marrow evaluation. The MDS duration in months were 17 and 12 respectively and the patients were transfusion dependent (Patient #1: two units every 2 weeks; Patient #2: two units every 3 weeks), refractory to erythropoietin (Patient #1: 80000 International Units/week; Patient #2: 40000 International Units/week plus G-CSF 150 µg/mg/day), and refractory to the Raza schedule (pentoxifilline, ciprofloxacin, amifostine and steroids). TNF-α levels were elevated (Patient #1: 114 pg/mL; Patient #2: 88 pg/mL with normal values 0-10). We have treated the patients with Infliximab at the doses usually used in active Crohn disease (5 mg/kg at day 0, 5 mg/kg at day +14, and 5 mg/kg at day +42). Hematologic status was recognized at day +21 and +49, and summarized in Table 1.

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day +14</th>
<th>Day +49</th>
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<tbody>
<tr>
<td>Hb g/dL</td>
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<td>8.2</td>
</tr>
<tr>
<td>WBC×10^6/µL</td>
<td>0.9</td>
<td>1.4</td>
</tr>
<tr>
<td>PMN%</td>
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<td>41</td>
</tr>
<tr>
<td>PLT×10^6/µL</td>
<td>54</td>
<td>43</td>
</tr>
<tr>
<td>Retic.×10^6/µL</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>TNF-α pg/mL</td>
<td>114</td>
<td>88</td>
</tr>
</tbody>
</table>

Both patients completed the treatment and were evaluated for toxicity and response. No adverse reaction was observed during infusion of infliximab, and serious infections were not observed during the treatment period or in follow-up. Response appear in pt 1 after the first dose but the maximum levels in Hb and WBC count were after the third dose. At the end of treatment the patients became transfusion-independent, sustained for 8 weeks; for this reason we have
included the patients in maintenance protocol (5 mg/kg every 8 weeks). Actually the transfusion care therapy is one unit every 30 day (mean). Infliximab is well tolerated and easily administered, and its use in combination with other drugs might be of benefit to some patients with MDS. A pilot trial to evaluated clinically-relevant responses is needed.

PU123
MYELODYSPLASTIC SYNDROME-RELATED LATENT AUTOIMMUNE HEMOLYSIS ENHANCED BY THE MITOGEN-STIMULATED DIRECT ANTIGLOBULIN TEST (MS-DAT)
Cesana C, Boschetti C, Barcellini W, Vercellati C, D’Avanzo G, Monguzzi A, Zanella A
*Unità Operativa Trasfusionale, Ospedale “Città di Sesto San Giovanni”; Sesto San Giovanni, **Dipartimento di Ematologia, IRCCS Ospedale Maggiore, Milano, and *Dipartimento di Ematologia, Ospedale Niguarda Ca’ Granda, Milano, Italy

Introduction: Myelodysplastic syndromes (MDS) can be associated with mild hemolysis due to ineffective erythropoiesis, acquired red blood cell (RBC) defects, paroxysmal nocturnal hemoglobinuria-like clones, or autoimmune hemolytic anemia (AIHA). Direct antiglobulin test (DAT)-negative AIHA is mostly diagnosed by exclusion. Although recombinant human erythropoietin (EPO) (rHuEPO) is effective in low-risk MDS, its safety in the presence of latent autoimmune hemolysis is largely unknown. We describe a MDS-related DAT-negative AIHA enhanced by rHuEPO and discovered by the mitogen stimulated (MS)-DAT, a recently described test able to detect latent anti-RBC antibodies in B-chronic lymphocytic leukemia. Case Report: An 80-year-old male was diagnosed as refractory anemia in May 2000. Peripheral blood (PB) showed hemoglobin (Hb) 6.6 g/dL, white blood cells 4.2×10^9/L (neutrophils 67%, lymphocytes 31%, monocytes 2%), platelets 87×10^9/L, MCV 109 fL, and Pelger-Huët-like anomaly. Bone marrow (BM) showed erythroid and megakaryocytic dysplasia, and normal myeloid precursors. The karyotype was 46 XY, add(9)(q34)[22]/22. At diagnosis reticulocyte counts were low (13×10^9/L), but increased lactate dehydrogenase (LDH) (985 U/L, normal: 270-600) and unconjugated bilirubin (1.71 mg/dL, normal: 0-0.7) and decreased haptoglobin (6 mg/dL, normal: 18-230) suggested mild hemolysis. The standard DAT and the indirect antiglobulin test (IAT) were repeatedly negative. Urine hemosiderin was positive. The BM showed erythroblastosis with trilineage dysplasia. The standard DAT and the IAT were repeatedly negative. Ham and sugar water tests were negative, and the cyt fluorimetric assessment of CD55/CD59 was unremarkable. Hb electrophoresis and RBC osmotic fragility were normal. RBC enzymes and SDS PAGE analysis of RBC membrane proteins were normal. MS-DAT showed autologous RBC-bound IgG in unstimulated whole blood culture (312 IgG ng/mL); mitogen stimulation with PHA, PMA and PWM increased the autologous RBC-bound IgG to 608, 782 and 432 ng/mL respectively. The transfusion supply doubled during rHuEPO treatment, and returned to 2 packed RBC units every 15 d after drug suspension. Conclusions: Our case (i) demonstrates that in MDS-related DAT-negative AIHA rHuEPO effectiveness fails, as previously observed in DAT-positive AIHAs associated with systemic autoimmune disorders; (ii) shows that refractoriness to rHuEPO in such occurrences is likely to result from hemolysis of an expanded clonal RBC population sensitized by anti-RBC antibodies; and (iii) suggests that, although the evidence of a mild DAT-negative hemolysis is not an absolute contraindication towards the use of rHuEPO in MDS patients, the assessment of latent anti-RBC antibodies by means of the MS-DAT is mandatory.

PU124
THALIDOMIDE IN THE TREATMENT OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES OR SMOULDERING ACUTE MYELOID LEUKEMIA
Istituto di Ematologia e Oncologia Medica “Seràgnoli”, Bologna; Divisione di Ematologia, Azienda Ospedaliera “S.Orsola-Malpighi”, Bologna; Divisione di Ematologia, Azienda Ospedaliera S.Salvatore, Pesaro, Italy

From January 2000, 7 patients affected by myelodysplastic syndromes (MDS), or smouldering acute myeloid leukemia (AML), not eligible for standard chemothera-
A case report of a 77-year-old man with CMML who developed pleural and pericardial effusion and presented autoimmune manifestations has been observed. The patient was symptomatic for recurrent throat and urinary infections sometimes associated with fever during the evening and fatigue without cutaneous and mucosal hemorrhagic diathesis, lymphohadonemegaly and splenomegaly. The blood cell count revealed leukopenia (2200 leukocytes/mm³) with severe neutrophilia (neutrophils 7 per cent) and monocytosis (1140 monocytes/mm³), thrombocytopenia (67000 platelets/mm³) while only a mild reduction of hemoglobin level (10 grams per deciliter) was noted. IgG level was 4527 mg per deciliter (polyclonal). Routine blood culture for the most common bacterial, fungal and viral agent, were negative. Bone marrow aspiration revealed hypercellularity (about 80 per cent), active erythropoiesis, increased monocytes and megakaryocytes and blast cell below 20 per cent. Peripheral blood and bone marrow smears substantiated a diagnosis of CMML. Karyotype was normal (46 XY). On molecular genetic analysis, no bcr/abl rearrangement was detectable (by RT-PCR). Some weeks later, the patient was admitted to our hospital because of a progressive dyspnea, fatigue, fever, dry cough. On examination, the patient was tachypnoic and pale; pulmonary examination revealed decreased bilateral breathing sounds with percussion dullness in both lungs. A chest radiography and computed tomography indicated bilateral pleural effusion (greater on the left), initial pulmonary fibrosis and cardiomegaly: a small pericardial effusion was confirmed by echocardiography. There was moderate splenomegaly without hepatomegaly and lymph node enlargement. The blood cell count was stable as to diagnosis. Thoracentesis was performed and revealed serous fluid. A cytological study of the fluid did not indicate a leukemic infiltration. The patient started antibiotic and antimycotic therapy for one week without clinical improvement. Immunological study of blood showed antinuclear positive antibodies (ANA) at a titer of 1:320 and anti-dsDNA positive antibodies, cytoplasmic anti-neutrophils positive antibodies (ANCA) with pattern p-ANCA, antiplatelet positive antibodies (PAIg), anticycdiolipin negative antibodies. Prednisone treatment was initiated (25 mg/day). Fever ceased immediately and progressive clinical improvement was observed. Two weeks later chest radiography showed the regression of the pleural effusion. The blood cell count revealed a resolution of leukopenia, neutropenia, thrombocytopenia and anemia. The patient is now on prednisone therapy and remains in good general health. Conclusions. Only 8 cases of pleural effusion and 7 cases of pericardial effusion during CMML have so far been described: 5 of 8 have been associated with pleural effusion due to leukemic infiltration while the 7 cases of pericardial effusion were all due to leukemic infiltration. Abnormal B- and T-cell interactions and deficient T-cell
responses to antigen presentation may play an important role in the pathogenesis of immune dysregulation.

PU126
ACQUIRED STORAGE POOL DISEASE: FIRST SIGN OF MYELODYSPLASTIC SYNDROME. A CASE REPORT.
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A 73-year-old male patient affected by a chronic myelomonocytic leukemia (CMML) is described. In July 2002 he presented a large abdominal hematoma consequent to physiotherapy. The anamnesis was negative for hemorragic disorders and he was sent to the Hematology Institute to be studied for a suspected acquired coagulopathy. At this time the patient had normal blood count and coagulation parameters. A prolonged bleeding time (14 min) was the only alteration observed. These data were very similar in three different examinations executed in our Institute. A von Willebrand Disease was excluded on the basis of the negativity of the screening tests: F VIII:C 118%; vWF 80%; CBA 100%; CBA/VWF 1.2. A platelet dysfunction was thus considered. Platelet aggregation (PA) revealed an impaired response to Collagen (5 µg/mL), arachidonic acid (1 mM), Epinephrine (15 µM) and ADP (5 µM). Platelet secretion of the dense granule contents was evaluated by means of the luciferin/luciferase system. The results of the ATP released obtained in response to the same agonists, at the same concentrations, showed the absence of granule secretion. This finding was consistent with an acquired storage pool disease. Electron microscopy studies confirmed a marked reduction of both α and, more evidently, dense granules constituents. Platelet agglutination following different concentrations of Ristocetin (0.75, 1, 1.25 and 1.5 mg/mL) (RIPA test) was normal, indicating no concomitant Bernard Soulier-like functional defect. The platelet count, as well as the mean platelet volume (MPV), remained within the normal range throughout the follow-up. Morphological examination confirmed the absence of alteration of the platelet size. On April 2003 the patient showed for the first time WBC 33,070×10⁹/L with 17.6% of monocytes (M 5,820×10⁹/L). A bone marrow aspiration and biological studies confirmed a diagnosis of chronic myelomonocytic leukemia. These observations provide further evidence that an acquired platelet dysfunction might represent the first sign of a myelodysplastic syndrome. Patients with acquired platelet defects require a close follow-up in order to early identify the eventual onset of myelodysplastic syndromes.

PU127
PROGNOSTIC SIGNIFICANCE OF BONE MARROW BFU-E AND CFU-GM IN PRIMARY MYELODYSPLASTIC SYNDROMES (MDS)
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The myelodysplastic syndromes (MDS) are clonal disorders of the bone marrow in which the capability of stem cells to differentiate is impaired and in which there is the possibility of evolution in acute leukemia. The necessity to identify prognostic factor loaded to classification that try to compare clinical data to biological results. Cell cultures (BFU-E, CFU-GM) in the past were utilized to identify prognostic class but without great confirmations. In this study we utilized bone marrow BFU-E and CFU-GM at presentation in MDS patients with the aim to correlate the results of hematopoietic cell cultures with diagnostic/prognostic classifications (FAB and IPSS). The patients were 26 (15 males, 11 females), median age 58.8 yrs (range 21-89): 8 RA, 5 RARS, 4 CMML, 9 RAEB. IPSS was applied when possible: 5 low risk, 7 int. 1, 3 int. 2. The numbers of BFU-E and CFU-GM were respectively 11±21 and 292±336x10⁶ cells in RAEB, 1±2 and 95±92 in CMML, 59±53 and 116±119 in RA/RARS. Because the absolute colony numbers with high standard deviations were inconclusive we tried to identify a new variable to better fit our results. The BFU-E/CFU-GM ratio seems suitable for our aim and moreover this new index appears to be well correlated to the clinical developments of our patients. With a cut off of 0.5 (high risk MDS were <0.5; low risk were >0.5) this ratio resulted significantly correlated to FAB classification (r=0.56) and well correlated to IPSS. Cell culture values fitted as BFU-E/CFU-GM ratio are useful to confirm or ameliorate the indications of others prognostic classifications.

PU128
MYELODYSPLASTIC SYNDROMES (MDS) WITH NORMAL KARYOTYPE: IDENTIFICATION OF CLINICAL FEATURES OF PROGNOSTIC SIGNIFICANCE FOR RISK OF TRANSFORMATION
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We retrospectively analyzed our series of MDS with normal karyotype observed in a period of 11 years, with the aim of identifying presenting clinical features of possible prognostic significance for poor outcome. Between January 1986 and December 1997, 98 patients were
At bone marrow morphological analysis, 55 × group of MDS patients. Studies are warranted to better characterize this sub-prognosis. More refined and accurate biological identifying MDS patients with normal karyotype at inferior prognostic value, clinical variables might be of help in detection of cytogenetic and/or molecular findings of best prognostic indicators for survival. In the absence of sis and morphologic FAB subgroup appeared to be the presence of hemorrhagic symptoms at time of diagno-

We treated 28 patients with low risk MDS (7 RA, 10 RARS, 11 RAEB), low endogenous erythropoietin (less than 500 mU/mL) as a part of the Italian Fatigue-

A CASE OF MYELODYSPLASTIC SYNDROME EVOLVING INTO ATYPICAL CHRONIC MYELOID LEUKEMIA

Introduction. Atypical chronic myeloid leukemia (aCML) is a rare myeloproliferative disorder characterized by leukocytosis, absence of Philadelphia chromosome or BCR-ABL rearrangement, marked dysplasia and poor outcome. According to FAB cooperative group, aCML can be distinguished from chronic myeloid leukemia (CML) and chronic myelomonocytic leukemia (CMML) by the combination of low basophil count and a percentage of monocytes ranging from 3-10% with markedly dysplastic immature granulocytes. In the present report, we describe the case of a man with myelodysplastic syndrome (MDS) who developed an aCML during the course of the disease. Case Report. A 69-year old man was admitted to our Department in May 1999 because of neutropenia (1.0 × 10^9/L); white blood cells were 3.0 × 10^9/L, hemoglobin was 12.8 g/dL with normal MCV, MCH and MCHC and platelet count was 191 × 10^9/L. Clinical examination was normal and a bone marrow aspirate showed marked dysplasia with a very low blasts count. These data supported a diagnosis of refractory anemia. So the patient was started to a follow-up program till October 2002 when a leukocytosis (24.9 × 10^9/L) and a splenomegaly appeared. Blood smear showed a high percentage of immature gra-
nocytes and a leucocyte alkaline phosphatase deficiency was revealed. A bone marrow aspirate showed red cell hypoplasia, myeloid hyperplasia with dysgranulopoiesis and both cytogenetic and BCR-ABL rearrangement analysis resulted negative, suggesting the diagnosis of aCML. Because of poor performance, the patient was treated only with hydroxyurea obtaining no satisfactory response and six months later he died from respiratory complications. Discussion. Morphologic features of aCML suggest that this disorder should be considered a distinct entity more related to MDS than to myeloproliferative disorders. The evolution of MDS into aCML has been well documented in literature and, although the heterogeneity of the reported cases, it is constantly characterized by a poor response to any chemotherapy regimen and short survival, as observed in our patient. Such evolution still remains unclear: aCML has been hypothesized to be an unusual transformation of myelodysplasia in which myeloid maturation becomes proliferative rather than ineffective. Since aCML represents a severe complication of MDS as well as the more frequent acute leukemia it would be interesting to find some clinical and/or laboratory parameters (leucocyte alkaline phosphatase pattern, cytogenetic analysis i.e.) able to predict such transformation so as to treat in a different way such patients.

References


PU131

BIOLGICAL EFFECTS OF TREATMENT WITH THALIDOMIDE IN MYELODYSPLASTIC SYNDROMES


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The antiangiogenic, immunomodulatory and anticytokine activities of thalidomide justify its use in myelodysplastic syndromes (MDS), since an abnormal bone marrow microenvironment may play a role in the pathophysiology of these disorders. However, the precise mechanism by which thalidomide acts in MDS is not yet fully clear. We treated with thalidomide at the dose of 50-200 mg daily on a compassionate basis seven patients with primary MDS (four RA, two RARS and one RAEB-t), M/F 3/4, median age 69 years (range 57-78), with serious or symptomatic cytopenia, who had not responded to previous therapies. Clonal cytogenetic abnormalities were present in three patients (5q- in one case, complex anomalies in two cases). Our aims were to verify its possible therapeutic efficacy and to evaluate its influence on some biological parameters. All patients but one were treated for at least 6 months and biological studies were performed before therapy and at intervals of 3 months thereafter. Treatment was associated with expected side effects, including constipation and weakness, in all cases. We observed reduction of the transfusion requirement in two cases (one RA and one RARS) within 2 months; both patients are still receiving the drug and maintain erythroid response after periods of treatment of 9 and 12 months respectively. One RA case became transfusion-independent 1 month from start, but he discontinued the treatment because of skin rash and relapsed 2 months after. Neutrophil and platelet values did not vary significantly. Significant reduction of bone marrow blasts from 25% to 9% was observed in the RAEB-t case. No additional chromosomal anomalies appeared during treatment. Increased BFU-E, CFU-E and CFU-GM were observed in three cases. The apoptotic rate of bone marrow cells diminished significantly in five cases from a mean of 50% (range 20-68) to a mean of 19% (range 8-36), whereas the proliferative activity did not change. Serum bFGF, TNF-α, IL-1β, IL-2 and IFN-γ levels did not vary significantly, whereas serum levels of IL-6 and VEGF tended to increase. Thalidomide treatment did not modify the expression of VEGF in bone marrow cells nor did it alter the expression of matrix metalloproteinases 2 and 9. A tendential increase of T suppressor lymphocytes as well as of NK cells was observed in the peripheral blood. In conclusion, our findings show that thalidomide may produce a fairly good hematologic improvement in erythroid series in MDS, with demonstration in vitro of the stimulation of hematopoiesis and with morphological evidence of reduction of apoptosis in bone marrow cells. This therapeutic effect of thalidomide does not seem to be mediated by inhibition of cytokine expression.
RECOMBINANT HUMAN ERYTHROPOIETIN (RHU EPO) FOR THE TREATMENT OF MYELODYSPLASIA AND ACUTE MYELOID LEUKEMIA WITH ERYTHROID MARROW EXPANSION (AML-M6)

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Erythropoietin is the lineage-specific hormone required for red cell production, influencing differentiation, proliferation and survival of bone-marrow committed erythroid progenitor cells. Recently, several studies have reported beneficial effects of treatment with rHuEPO in myelodysplasia and in anemia of chemotherapy-treated patients suffering from non-Hodgkin's lymphomas, multiple myeloma and solid neoplasms. We report a single Institution experience on the use of rHuEPO in patients suffering from non-Hodgkin's lymphomas, multiple myeloma and solid neoplasms. We report a single Institution experience on the use of rHuEPO in patients suffering from myelodysplastic (SM D) syndromes (RA, RARS, RAEB I) or acute myeloid leukemia with erythroid hyperplasia (AML-M6). From January 2001 to January 2003, 40 patients, 30 males and 10 females (RA: 20; RARS: 7; RAEB I: 6; AML-M6: 7), median age 75 years (range 48-90), received rHuEPO by sc injection at the dose of 10 MIU 3 times/week (patients with MDS) or 5 times/week (patients with AML-M6). Informed consent was obtained from all patients. At beginning of the therapy, median Hb level and median PCV were 8.3 g/dL and 25%, respectively, and serum EPO level was <200 mU/mL, with a reduced O/P ratio in 38 cases. Patients with AML-M6 received a differentiative/maturative approach with the association of rHuEPO and G-CSF because they were not eligible for intensive chemotherapy due to age, comorbidities or to leukemia persistence after two lines of cytotoxic treatment. All of them had the subtype of AML-M6 called ‘with maturation’ (i.e., blastosis with erythroid bone marrow and dyserythropoiesis). After 12 weeks, increased hemoglobin level (median 10 g/dL) was observed in 16/20 (80%) patients with RA, in 4/7 (57%) of those with RARS and in 2 cases (33%) with RAEB I. As for AML-M6 patients, complete remission of leukemia was achieved in 5/7 cases, including one patient with chemotherapy refractory disease. In the subset of responder patients, CR was observed after four weeks from the start of therapy and was associated with a full blood count recovery. Remission duration lasted from three to six months, and was associated with an excellent quality of life. Two patients died of disease progression; one patient died in remission because of infection. One responding patient is alive with stable disease after six months from the start of the therapy. While confirming the beneficial effect of rHuEPO in a majority of patient with MDS without excess of blasts, we would like to stress the possibility of achieving a transient CR in a proportion of patients with AML-M6 with maturation, a disease known to be resistant to standard chemotherapy.
**MYELOMA AND PLASMA CELL DYSCRASIAS**

PU133

**EXTRAMEDULLARY RELAPSE AND PROGRESSION IN MULTIPLE MYELOMA PATIENTS RECEIVING THALIDOMIDE: A DEBATE ON A POSSIBLE RELATIONSHIP**


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Thalidomide (thal) plus dexamethasone (dex) is a salvage treatment in multiple myeloma (MM), allowing to achieve a response in an about one third of cases. Intravenous zolendronic acid provides a meaningful therapeutic effect to skeletal disease, reducing pain and vertebral fractures and being maybe able to exert direct anti-myeloma effects by its immunomodulating effects. The combination of these three agents represents the most innovative current salvage approach in the management of refractory and relapsed pre-treated patients with MM. However, even in responders to salvage therapy, the relapse is unavoidable and the patients can only benefit of supportive measures. The pattern and the outcome of relapsed/refractory disease in 21 consecutive patients (8 M/13 F, median age 74 yrs.) with pre-treated advanced MM receiving a salvage treatment with thal, dex and zolendronate, are reported. Thal was given at a median dose 150 (100-400) mg/day, dex and zolendronate were respectively given at the dose of 40 mg/day I.V or P.O. for 4 days and of 4 mg as single I.V. infusion both every 4 weeks. Eight out of 21 (38%) patients showed a stable disease, 5 (24%) progressed and 8 (38%) had a complete or partial response. Out of the 8 responders, one deceased without relapse (congestive heart failure) and 7 progressed. Median duration of response was 8 (2-12) months. To date, 4 patients are alive: 3 with stable MM and 1 with progressive disease. The median overall survival was 8 (1-16) months. Out of the 7 patients experiencing relapse, 3 cases presented untreated and very extended pulmonary (2) and frontal bone (1) plasmocytomas. The remaining 4 patients presented progressive increase of paraprotein levels associated with plasmacytic BM infiltration and new osteolytic lesions. The extramedullary manifestations were treated with radiotherapy and in all relapsers a weekly dose (500 mg) of cyclophosphamide was added to the current therapy. All relapsed patients died after 2 (1-3) months. Out of the 5 patients with disease progression, 4 presented the typical hematologic features and 1 a large plasmacytoma, extended from the 5th lumbar spine to the iliac region. This patient, alive from 19 months, received radiotherapy, weekly cyclophosphamide and regular courses of dex, as above reported, achieving a partial and shortly maintained control of disease (5 months) and then progressed, presenting extended multiple plasmocytomas involving the right lung, multiple ribs and vertebral spine. A treatment with melphalan I.V. 25 mg/spm with palliative intent was started but she progressively deteriorated. Median survival after progression was 2 (1-19) months. The extramedullary spread, found in 4 (33%) out of 12 refractory/relapsed patients, suggests a thal-induced selection of a resistant and more aggressive clone, lacking the regulatory adhesion molecules, involved in the plasma cells traffic and homing. Studies on larger MM series of patients treated with thalidomide are required to establish whether our observation may be due a coincidental events or correlated to a distinctive pathological pattern of disease progression, that could be associated with this treatment.

PU134

**TERATOGENICITY PREVENTION IN THALIDOMIDE TREATMENT IN MULTIPLE MYELOMA**

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Discrete changes in regulation of gene expression in multiple myeloma cells demonstrate that thalidomide and second generation-analogs may work as a modulator of tumor necrosis α (TNF α). The use of thalidomide represents a useful treatment for MM and, in cases of resistance to the drug, in association with PS 341 in far advanced MM (Barlogie, 2002). Known as the greatest drug tragedy of our time, thalidomide is back in the international spotlight, not only for recently discovered important therapeutic properties, but also for the Risk Management Programme in which thalidomide will be prescribed, dispensed and used only if physicians, pharmacists and patients register. The PRMP, abbreviation for Pharmion Risk Management Programme, provides fundamental additional value, since it will work as an effective means of risk management aimed at preventing teratogenicity in thalidomide treatment of MM. Thalidomide approval in MM treatment will be an important step forward both safeguarding patient’s choices and implementing a well-defined program for management, education and responsibleness of patients. The latter is an effort to prevent the risk of teratogenicity of the drug. Once again in the history of thalidomide, all the questions regarding the relationships between health authorities and pharmaceutical companies, physicians, pharmacists and patients, are directed towards the need for a fully developed and evolved drug policy able to suc-
cesfully implement thalidomide’s approval. Therefore, PRMP arises from the requirements of managing the distribution of a potentially unsafe drug, avoiding the dangerous black market of thalidomide’s import from countries in which is available and, finally, monitoring all the physicians, pharmacists and patients involved, so that they strictly fulfill procedures of the program: registration of physicians, pharmacists and patients; education specifically addressed to each of those categories in order to comply with the PRMP and informed consent of patients.

PU135
DIAGNOSTIC APPROACH AND CLINICAL OUTCOME OF SPINAL PLASMA BLASTIC MYELOMA


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Plasmablastic myeloma of the spine is a rare entity (<10% of Myeloma cases), usually characterized by both a lack of typical hematologic and serum features of plasma cell dyscrasia and poor prognosis. An early neurological approach is mandatory to make a correct diagnosis and to establish the therapeutic strategy. Between June 2001 and October 2002, 5 patients (4 males/1 female, median age 54 yrs, range 39-64 yrs), were admitted to Emergency Department of our Hospital for acute vertebral bone pain. Spinal column NMR and CT scan images showed the presence of aggressive lytic bone lesions at C1, D6, L3 levels, however 3 of the patients had more than one bone lytic lesion. The skeletal X-ray did not evidence the involvement of other segments. Peripheral blood and bone marrow (<10% atypical plasma cells) parameters were not diagnostic for multiple myeloma (MM): only 2 pts. had a serum M component (IgG<2 g/dL), but in no case were Bence Jones protein and hypogammaglobulinemia detectable; serum β2-microglobulin and reactive C protein were increased in 2 and 1 pts, respectively; whereas serum Ca++ and creatinine were in the normal ranges. Since no patients fulfilled MM diagnostic criteria, a neurosurgical approach was planned. During surgery both vertebral biopsy and stabilization (2 cases) were carried out. On the histologic specimens basis the diagnosis of plasmablastic/anaplastic MM G3 grading) and immature MM G2 grading) was done in 4 and 1 pts., respectively. One patient died soon after neurosurgery because of massive pulmonary embolism, the remaining 4 were enrolled in VAD regimen (4 cycles): 3 resulted responders (2 complete and 1 partial) and one was refractory. This one died 11 months later in active disease. Of the 3 responders, 1 underwent autologous peripheral stem cell transplant, 1 tandem autologous peripheral stem cell transplant, 1 (64 yrs old) non-myeloablative allogeneic transplant; to date they are alive in complete response for 22+, 11+, 10+ months. The onset of hematologic neoplasia in this cohort of patients was characterized by dramatic symptoms, but lack of biophysical and marrow diagnostic criteria, probably due to the poor differentiation of the plasma cells tumor. An early neurosurgical approach plays a role of choice to make the differential diagnosis as well as to start, as soon as possible, tailored aggressive therapy.

PU136
COMPLETE CONTINUING REMISSION IN REFRACTORY MULTIPLE MYELOMA AFTER THE USE OF ZOLEDRONATE


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Multiple myeloma is a neoplastic disease, that affects especially the elderly, even if in recent years it has also been observed in young patients. Despite the progresses in the therapy, it remains an incurable disease. Painful osteolytic bone destruction is a frequent complication of multiple myeloma, that affects patient’s quality of life. Bone destruction is mediated by normal osteoclasts, which respond to local osteoclast activating factors (OAF), as TNFβ, RANK ligand IL-1β, e IL6, TNFα, parathyroid hormone-related protein (PTHrP), MIP-1-α, produced by myeloma cells or other cells of the microenvironment. Bone-loss leads to fractures, spinal cord compression, hypercalcemia and bone pain. Bisphosphonate therapy has been shown to reduce complications of bone lesions in multiple myeloma and in other malignancies. In fact bisphonates act on osteoclasts to inhibit excessive bone resorption. Recent reports have demonstrated in vitro and in vivo bisphosphonate effects; in particular it seems that Zoledronate, a new generation bisphosphonate, also exert antitumor effects on myeloma cells; this drug has cytotoxic capacity because it causes apoptosis and proliferation block. We report the case of a 55 year old female patient, affected by multiple myeloma IgGλ, without osteolytic lesions, since September 1999. She had previously been treated with conventional chemotherapy as V.C.M.P. schedule (vincristine, cyclophosphamide, melphalan, prednisone), obtaining complete remission (CR). Subsequently, in April 2002, bone marrow plasmocytosis was about 20%. For the relapse, she was treated with thalidomide, dexamethasone and zoleondrate. A dose of Thalidomide of 200 mg/d per os was given at bedtime; 20 mg/d of dexamethasone was dosed for four days every 3 weeks; 4 mg of zoleondrate was given as a single i.v. 15-min infusion every 3 weeks. After 2 months, due to lethargy, hypertension and abdominal pain, she interrupted thalidomide and dex-
amethasone, continuing only the zoledronate infusions, without any toxicity. After five months, the patient showed a significant response; a greater than 75% reduction of marrow plasmocytosis (bone marrow plasmocytosis was about 4%) was observed and the para-protein completely disappeared at immunofixation. Currently, after a year of zoledronate therapy (17 zoledronate 4 mg infusions), the patient is in complete remission, showing a bone marrow plasmocytosis of about 3%. Using thalidomide it is possible to obtain a complete remission, but our case demonstrates that zoledronate is an important drug for multiple myeloma therapy. In addition to its well-known capacity on bone marrow resorption, this drug seems to have a direct anti-tumor effects on multiple myeloma cells. Recent reports have shown the effects of bisphosphonate zoledronate on both multiple myeloma cells and bone marrow stromal cells, inducing apoptosis and inhibition of IL6 and MMP-1, the major metalloproteinase involved in bone resorption. So our patient has obtained a complete remission with only zoledronate therapy, without toxicity, demonstrating it as a safe and well tolerated drug. These data needs to be confirmed in larger clinical trials.

PU137
CRITICAL EVALUATION OF THE ROLE OF TECHNETIUM-99M-SESTAMI B I SCINTIGRAPHY IN STAGING AND FOLLOW-UP OF MULTIPLE MYELOMA, BY COMPARISON WITH MNR
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In our study we evaluated the role of SestaMibi scintigraphy in myeloma (MM) and verified the presence of bone lytic lesions by MNR. Methods. Forty-five patients (30 at baseline, 15 at follow up) affected by MGUS (6), MM (38: stage I-8 pts, stage II-10 pts, stage III-19 pts) and Waldenström's macroglobulinemia (1) were studied by whole bone scans obtained after administration of 99m Tc SestaMibi. Results are expressed as pattern of SestaMibi distribution (normal, diffuse, focal, diffuse + focal) and as score of uptake extension (1-3) and intensity (1-4) summed up. Ten pts with D pattern and maximum score <= 4 were evaluated with spine RMN too. Results. Baseline SestaMibi results correlate with stage (see Table below) and main prognostic factors. In the pts evaluated at follow up the average score was different according to disease activity (1,1 vs 4). Pts with κ chain MM had a more advanced stage and higher score than pts with λ chain MM and were the only showing either F or D+F pattern. No correlation was found between heavy chain and stage or SestaMibi results. No difference was found in average SestaMibi score at baseline between resistant and responsive to treatment pts, (4,1 vs 4), whereas a difference has been observed between pts with aggressive disease (progression or early relapse) and pts with long lasting remission or stable disease (4,9 vs 3,3). After chemotherapy the average score did not change in resistant pts while it decreased from 4,3 to 1,5 in pts achieving clinical remission (in 4 pts SestaMibi converted to negative, while conventional x-Ray skeletal survey was positive). At relapse SestaMibi converted to positive. In ten pts with D pattern and maximum score <= 4 spine MNR did not show any clear sign of bone involvement. Conclusions. Our study confirms the correlation between SestaMibi and stage, prognostic factors, disease activity and clinical status in MM also reported in previous studies. Different results in kappa and lambda chain MM had not been reported in the literature before; further investigation of these data is therefore needed. The higher score at baseline in pts with poor prognosis could represent an indication to more intensive treatment. Spine MNR results in ten pts show that a diffuse SestaMibi pattern with low score (max. 4) is due to bone marrow plasma cells uptake and not to bone infiltration with lytic lesions; so it does not signal a change in stage neither it is an indication to early beginning of therapy. We plan to evaluate MNR images in pts with diffuse pattern and high score and in pts with focal pattern. Our data confirm the role of SestaMibi in prognostic evaluation and follow up, but not its relevance in staging.

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thrombosis (DVT) have been reported at various incidence according to the use of thalidomide as single agent (5%) or in combination with Dexamethasone (10-15%) or chemotherapy (30%). The pathophysiology of this complication is not yet clear. Previous studies suggest that the increased risk might be attributed to an endothelial damage by thalidomide. We performed on a cohort of 13 relapsed-refractory MM patients treated with low dose Thal (100 mg/day) and Dexamethasone (20 mg p.o./die for 4 days every two weeks) serial evaluations of different laboratory parameters to follow their variations during the treatment. No patients showed previous history of thrombosis. In the first three months of treatment only one out of 13 patients (7%) developed symptomatic DVT, involving bilaterally the tibial district and associated with non fatal pulmonary embolism. Thrombosis arise in the first month after starting Thal /Dex. We assayed baseline laboratory evaluation for inherited risk factors for thrombosis: abnormalities of prothrombin gene (G20210A), MTHFR gene (C677T and A1298C) and factor V Laiden; three patients had a homozygosis for the same mutation, but without evidence of factor V Laiden (G1691A). No patients had abnormalities of prothrombine gene; one showed a heterozygosis for factor V Laiden; three patients had a homozygosis for MTHFR gene mutation and three patients a heterozygosis for the same mutation, but without evidence of hyper-homocysteinemia before or during therapy. The only one patients who had thrombosis was heterozygous for mutated gene MTHFR C677T, with persisting normal value of homocysteine. We evaluated at baseline and after 1, 2 and 3 months the levels of plasma anti-thrombin III, protein C, protein S, and activated protein C resistance (APCR). Only one patient had deficit of anti-thrombin III, two of a deficit of protein C, no one showed deficit of protein S neither APCR: these parameters were never significantly modified by Thal-Dex therapy in all patients. The only patient who developed DVT did not present deficit of these parameters. Since plasma thrombomodulin, PAI I (plasminogen activator inhibitor I) are thought to be of value as marker of endothelial injure and prothrombin fragments 1+2 markers of hemostatic activation, we investigate their plasma concentration according the same schedule (0, 1, 2, and 3 months). Only thrombomodulin (TM) varied significantly, showing an initial decrease in the first month (p=0.02) and a successive normalisation at three months. In particular, the patient who experienced DVT showed as well a decrease of TM from the baseline value of 59.26 ng/mL to 10.90 ng/mL after 1 month.

In conclusion, we did not find relevant abnormalities of the laboratory assays correlated with thalidomide therapy. The only significant observation was a decrease of thrombomodulin in the first month of therapy.

PU138
EVALUATION OF MARKERS OF HYPERCOAGULABLE STATE AND ENDOTHELIAL INJURY IN MULTIPLE MYELOMA (MM) PATIENTS TREATED WITH THALIDOMIDE AND DEXAMETHASONE (THAL-DEX)
Lorenzi A, Corso A, Terulla V,* Airoti F,* Varettoni M, Manniagacavalli S, Zappasodi P, Rusconi C, Lazzarino M Division of Hematology, University of Pavia, *Department of Microbiology, IRCCS Policlinico San Matteo, Pavia, Italy

In Table 1 we reported mean and median values of TM at the serial evaluations.

<table>
<thead>
<tr>
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<th>Median (ng/mL)</th>
<th>Mean (ng/mL)</th>
<th>Standard deviation</th>
</tr>
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<tbody>
<tr>
<td>Baseline</td>
<td>21.04</td>
<td>23.54</td>
<td>18.76</td>
</tr>
<tr>
<td>After 1 month</td>
<td>10.98</td>
<td>10.54</td>
<td>5.30</td>
</tr>
<tr>
<td>After 2 months</td>
<td>16.46</td>
<td>16.70</td>
<td>9.40</td>
</tr>
<tr>
<td>After 3 months</td>
<td>11.90</td>
<td>21.35</td>
<td>10.21</td>
</tr>
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</table>

The role of Helicobacter pylori (H. pylori) in the incidence and clinical course of monoclonal gammopathy of undetermined significance (M GUS) is matter of debate. Recently A.A. Malik (Am J Gastroenterol, 2002; 97:1371) has found a proportion of M GUS patients infected by H. pylori who showed disappearance of gammopathy upon eradication of H. pylori. S.V. Rajkumar (Br J Haematol, 2002;119:706) did not confirm either the incidence and the resolution of M GUS with H. pylori therapy. We have performed a study on 32 consecutive newly diagnosed M GUS patients. Diagnosis of M GUS was made according to 1) serum monoclonal protein level <3.5g/dL (IgG), <2.0g/dL (IgA), and/or urine M component <1g/24h 2) bone marrow plasma cells<10% 3) normal serum calcium, hemoglobin level and serum creatinine, no clinical or laboratory features of amyloidosis or light chain deposition disease. Breath urea test was added to the routine analysis to ascertain H. pylori infection, confirmed with endoscopic histology. Ten of 32 (31%) patients had H. pylori infection. Of these 10 no patients showed modification of gammopathy after disappearance of the H. pylori. In conclusion, the rate of H. pylori infection in M GUS compares favourably with non M GUS population and in our series no patients showed resolution of M GUS with H. pylori infection.

PU139
HELICOBACTER PYLORI INFECTION AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE
pu140
Thalidomide and Dexamethasone for Poor Prognosis Multiple Myeloma Patients
Azienda ULSS 12 Veneziana, Venezia, Italy

Twenty-three poor prognosis multiple myeloma patients underwent thalidomide plus dexamethasone because of relapsed or refractory multiple myeloma. Seventeen of the 23 (74%) had received autologous transplant, 15/23 (65%) double, 2/23 triple transplant. The median treatment lines was 4 (2-8). The therapy consisted of thalidomide, initial dose of 200 mg/d with 200mg increments at two weeks intervals to a maximum tolerated dose, up to 800mg. Dexamethasone 40 mg/d on day one through 4, monthly. In a median time 3.5 months (1-8), 15 patients out of 23 (65%) exhibited partial response. With a median follow-up of 26 months, the time to treatment failure was of 16 and the response duration of 10.5 months. Median survival from starting therapy was 18 months. The median administered dose was 400 mg/d. Grade 3 neuro toxicity was recorded in 2 patients requiring reducing dosage. No patients had grade 3 hematotoxicity. Deep vein thrombosis occurred in 2 patients. In conclusion, our study confirms that combination of thalidomide plus dexamethasone achieves high response rate, even in heavily pretreated multiple myeloma patients.

pu141
Clinical Improvement after Autologous Stem Cell Transplantation in Three Patients with AL Amyloidosis
Clinica Ematologia, Dipartimento di Ricerche Morfologiche e Cliniche, Policlinico Universitario di Udine, Italy

High-dose chemotherapy with hematopoietic stem cell support is a promising treatment for patients with AL amyloidosis in order to improve organ function and prolong survival. Herein, we report the clinical course and outcome of 3 patients with AL amyloidosis treated with high-dose chemotherapy and autologous stem cell transplantation. Patient #1 was a 56-year-old woman with spontaneous purpura, macroglossia, carpal syndrome, hepatomegaly and serum IgG/κ monoclonal gammopathy and 30% plasma cell bone marrow infiltration. She was treated with 4 monthly cycles of VD chemotherapy and obtained a partial response. Patient #2, a 49-year-old woman, started with a nephrotic syndrome, micromolecular κ gammopathy and 85% plasma cell bone marrow infiltration. No response was seen after standard chemotherapy with 4 VAD cycles. Patient 3 was a 47 year-old woman, who suffered from dyspepsia and diarrhea, renal insufficiency and hepatomegaly; she had also a minimal IgG/κ monoclonal gammapathy and was refractory to 2 VAD cycles and to 2 melphalan plus prednisone courses. Peripheral blood stem cells were collected after cyclophosphamide + G-CSF at the dose of 7 g/m² (Patient #1 and 2) or 4 g/m² (patient 3). Conditioning treatment was carried out with either melphalan 120 g/m² and busulfan 16 mg/kg (Patient #1 and 2) or melphalan 140 g/m² alone (patient 3). Time between diagnosis and autotransplantation was 20, 12 and 11 months, respectively. All 3 patients had WHO grade III-IV mucositis and fever during aplasia period. Patient 3 also developed peripheral edema and diarrhea with fecal colture positive for Candida albicans. No renal toxicity was seen, except in patient 3 who had a moderate increase of creatinine. Long-term complications included two febrile episodes and transient thrombocytopenia in Patient #1 and herpes zoster in Patient #2 within 4 months after ASCT. At the 6th month evaluation, Patient #1 had reached a complete remission of her monoclonal disorder and 70 months after autotransplantation she continued to show improvement of macroglossia, purpura and normalization of alkaline phosphatase serum level. Patient #2 had a partial remission of monoclonal gammapathy with persistence of nephrotic syndrome. Forty-one months after ASCT she had a progression of amyloid disease with restrictive cardiomyopathy, congestive heart failure and orothostatic hypotension. A second autotransplantation was done, conditioned with melphalan 140 g². Hematologic recovery was fast and uneventful. Ten months after second autotransplantation the patient showed the disappearance of symptoms and signs of congestive heart failure and > 90% reduction of proteinuria. At the evaluation 15 months post-transplantation, patient 3 had no gastrointestinal symptoms, alkaline phosphatase serum level and liver size were normal and the creatinine concentration had fallen from 2 to 1.5 mg/dL. In conclusion, autologous stem cell transplantation in patients with AL amyloidosis appears to be an effective procedure that is worth extending to a large number of patients.

pu142
Low Dose Thalidomide±Dexamethasone Allows a Prolonged Control of Disease in the Majority of Patients with Multiple Myeloma
Ballerini F, Varaldo R, Canepa L, Clavio M, Miglino M, Venturino C, Balocco M, Michelis GI, Gobbi M
Department of Haematology and Oncology, University of Genoa, Italy

We report our long-term follow-up experience with relatively low-dose thalidomide (THAL) plus or minus dexamethasone (DEX) in MM patients. Twenty-nine
consecutive relapsed (#19), refractory (#6) and naïve (n. 4) MM patients were given THAL, at a starting dosage of 100 mg/day. The dose was increased by 100 mg every two weeks according to tolerance and response. In patients not responding to the maximum tolerated dose of THAL, 2-4 monthly courses of DEX (40 mg/day × 4 days) were associated. After the achievement of a stable response thalidomide dosage was progressively reduced to a minimum effective dose. Four patients discontinued therapy for disease-related complications before one month of therapy. Two patients have not been yet evaluated. Twenty-three patients received therapy for at least one month and were therefore evaluated for toxicity and efficacy. Males were 12, females 11, median age was 66 years (range 30-81). The monoclonal component (MC) isotype was IgG in 17 patients, IgA in 5, and IgD in 1. Stage was IA in 3 patients, IIA in 4, IIIA in 13, III B in 3. Nineteen patients had received a median of 2 regimens of chemotherapy: 6 of these had undergone high dose therapy with autologous stem cell rescue. Complete response (CR) required > 75% MC reduction; partial response (PR) a 50-75% MC reduction; minimal response (MR) a 25-50% MC reduction. The maximum effective or tolerated dosage of thalidomide was 100 mg in 10 patients, 200 mg in 6, 300 mg in 1, 400 mg in 5, and 600 mg in 1 patient. In 18 patients DEX was associated. Six out of 23 patients obtained CR (26.1%), 8/23 PR (34.8%), 6/22 MR (26.1%); 3/23 did not respond (13%). The overall response rate (OR) was 87%. The median response duration was 19 months (range 2-39). Eight out of the 20 responding patients progressed while on maintenance therapy, 1 stopped THAL because of pulmonary embolism, one died of gastrointestinal hemorrhage, and one underwent high dose therapy. With a median follow-up of 24 months (range 5-39), nine patients maintain response with 50 mg/day of THAL (25-100 mg). The majority of patients experienced minor side effects (sedation, somnolence, constipation and peripheral neuropathies), which did not lead to the withdrawal of therapy. During the treatment deep venous thrombosis was observed in 5 patients (2 of these had received only THAL) but led to the suspension of therapy in only one patient (because of pulmonary embolism). All patients who experienced deep vein thrombosis were responders. Two cases of impotence were reported but one patient (because of pulmonary embolism). All patients not responding to the maximum tolerated dose thalidomide dosage was progressively reduced to a minimum effective dose. Four patients discontinued therapy for disease-related complications before one month of therapy. Two patients have not been yet evaluated. Twenty-three patients received therapy for at least one month and were therefore evaluated for toxicity and efficacy. Males were 12, females 11, median age was 66 years (range 30-81). 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Six out of 23 patients obtained CR (26.1%), 8/23 PR (34.8%), 6/22 MR (26.1%); 3/23 did not respond (13%). The overall response rate (OR) was 87%. The median response duration was 19 months (range 2-39). Eight out of the 20 responding patients progressed while on maintenance therapy, 1 stopped THAL because of pulmonary embolism, one died of gastrointestinal hemorrhage, and one underwent high dose therapy. With a median follow-up of 24 months (range 5-39), nine patients maintain response with 50 mg/day of THAL (25-100 mg). The majority of patients experienced minor side effects (sedation, somnolence, constipation and peripheral neuropathies), which did not lead to the withdrawal of therapy. During the treatment deep venous thrombosis was observed in 5 patients (2 of these had received only THAL) but led to the suspension of therapy in only one patient (because of pulmonary embolism). All patients who experienced deep vein thrombosis were responders. Two cases of impotence were reported but the relation with THAL administration was uncertain. A marked improvement of Hb values (a median of 2.5 g/dL) was observed in all responding patients. In conclusion low dosage THAL±dexamethasone proved to be an effective and relatively safe therapy for advanced and non pre-treated MM patients. Furthermore our experience shows that a careful modulation of THAL dosage, on the basis of individual tolerability and response, may achieve prolonged responses without a relevant incidence of side effects.

**PU143 INFECTION COMPLICATIONS IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER HIGH DOSE MELPHALAN: A SINGLE CENTER RETROSPECTIVE ANALYSIS OF AN OUTPATIENT STRATEGY**


The principal complication in patients undergoing autologous stem cell transplantation (ASCT) is infection, which represents an impediment to outpatient based-transplants. We performed 133 ASCT in patients with multiple myeloma (MM), following either a total inpatient model (TIM, 56 cases) or outpatient model (OUT, 77 cases). Induction chemotherapy mainly consisted of DAV +/- high-dose CTX. All patients were conditioned with high-dose melphalan (HDM) (200 mg/m²). Cases were equally distributed for age, sex and disease status at transplantation. There were no significant differences between the two groups of patients with respect to the number of stem cells infused (5.9±5.5×10⁶/kg in TIM vs. 5.2±2.0×10⁶/kg in OUT), the time to granulocyte recovery (9.0±0.8 days in OUT vs. 9.0±0.9 in TIM) and the time to platelet engraftment (12.9±4.4 days in TIM vs. 12.7±2.1 in OUT). The main characteristics of infectious complications and mucositis score are detailed in Table 1.

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>TIM</th>
<th>OUT</th>
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<tbody>
<tr>
<td>No. of Pts.</td>
<td>56</td>
<td>77</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>44 (78.6%)</td>
<td>39 (50.6%)</td>
</tr>
<tr>
<td>No</td>
<td>12 (21.4%)</td>
<td>38 (49.4%)</td>
</tr>
<tr>
<td>Fever Duration (median, d)</td>
<td>3.09</td>
<td>1.63</td>
</tr>
<tr>
<td>Classification of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUD</td>
<td>25 (54.2%)</td>
<td>30 (76.9%)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>11 (25%)</td>
<td>2 (5.2%)</td>
</tr>
<tr>
<td>Gran+</td>
<td>10 (22.2%)</td>
<td>2 (5.2%)</td>
</tr>
<tr>
<td>Gran-</td>
<td>1 (10%)</td>
<td>-</td>
</tr>
<tr>
<td>PC/related</td>
<td>6 (13.2%)</td>
<td>3 (7.7%)</td>
</tr>
<tr>
<td>Gran+</td>
<td>6 (13.6%)</td>
<td>3 (7.7%)</td>
</tr>
<tr>
<td>Not valuable</td>
<td>6 (13.6%)</td>
<td>3 (7.7%)</td>
</tr>
</tbody>
</table>
showed also monosomy 13q14. We did not find any detected in 14/95 pts (15%) and among these pts 7 than 50% of plasma cells. t(11;14)(q13;q32) was while in 30 pts (77%) the deletion was present in more patients (41%). In 9 of this 39 pts (23%) the deletion was present in less than 50% of analysed plasma cells, and studied, comparing the traditional morphological and immunohistochemistry results with the molecular profile using interphasic FISH. Bone marrow samples were studied for morphological exam and for immunohistochemistry (κ and λ light chains, double staining of CD138/Mib 1), while an aliquot of fresh marrow blood was treated for positive selection using an anti CD138 monoclonal antibody linked to magnetic microBeads. This enrichment were then used for analysis of abnormalities on chromosome 13 [13q14 deletions (LSI D13S319)] and for t(11;14)(q13;q32) (LSI IGH/CCND1). We found monosomy 13q14 in 39/95 patients (41%). In 9 of this 39 pts (23%) the deletion was present in less than 50% of analysed plasma cells, while in 30 pts (77%) the deletion was present in more than 50% of plasma cells. t(11;14)(q13;q32) was detected in 14/95 pts (15%) and among these pts 7 showed also monosomy 13q14. We did not find any correlation between the proliferating plasma cells and deletion 13q14 or positivity for t(11;14). Moreover no relationship seemed to exist between t(11;14) and monosomy 13q14. We are investigating the prognostic relevance of the genetic alterations studied.

Overall, these data suggest that: 1) infectious complications are likely to occur in the outpatient group; 2) FUO is more frequent in the outpatient group; 3) there is no difference between the two groups in mucositis score. Of note, one death occurred in the inpatient group because of complications. In conclusion, although some data could be explained by biased selection, our experience confirms the feasibility of outpatient program. Ambulatory care, when compared with inpatient care, appears to have a low risk of infections and, probably, a better quality of life.

Funding: Partially supported by Regione Calabria and AIL.

PU144
INCIDENCE OF DELETION CHROMOSOME 13 AND t(11;14)(q13;q32) IN MULTIPLE MYELOMA USING INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION
Mura MA, Veronese S, Barbarano L, Morra E, Gambacorta M
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Multiple myeloma (MM) is a plasma cell disorder that shows biological and clinical heterogeneity with genetic alterations and an unfavorable prognosis (median survival 36 months). Deletion of 13q14 has been associated with unfavourable prognosis while there are still doubts about the prognostic significance of t(11;14) (q13;q32). Ninety-five patients with MM were evaluated and studied, comparing the traditional morphology and immunohistochemistry results with the molecular profile using interphasic FISH. Bone marrow samples were studied for morphological exam and for immunohistochemistry (κ and λ light chains, double staining of CD138/Mib 1), while an aliquot of fresh marrow blood was treated for positive selection using anti CD138 monoclonal antibody linked to magnetic microBeads. This enrichment were then used for analysis of abnormalities on chromosome 13 [13q14 deletions (LSI D13S319)] and for t(11;14)(q13;q32) (LSI IGH/CCND1)]. We found monosomy 13q14 in 39/95 patients (41%). In 9 of this 39 pts (23%) the deletion was present in less than 50% of analysed plasma cells, while in 30 pts (77%) the deletion was present in more than 50% of plasma cells. t(11;14)(q13;q32) was detected in 14/95 pts (15%) and among these pts 7 showed also monosomy 13q14. We did not find any correlation between the proliferating plasma cells and deletion 13q14 or positivity for t(11;14). Moreover no relationship seemed to exist between t(11;14) and monosomy 13q14. We are investigating the prognostic relevance of the genetic alterations studied.

Overall, these data suggest that: 1) infectious complications are likely to occur in the outpatient group; 2) FUO is more frequent in the outpatient group; 3) there is no difference between the two groups in mucositis score. Of note, one death occurred in the inpatient group because of complications. In conclusion, although some data could be explained by biased selection, our experience confirms the feasibility of outpatient program. Ambulatory care, when compared with inpatient care, appears to have a low risk of infections and, probably, a better quality of life.

Funding: Partially supported by Regione Calabria and AIL.

PU145
THALIDOMIDE AND PERCUTANEOUS VERTEBROPLASTY IN RELAPSED-REFRACTORY MULTIPLE MYELOMA: REPORT OF A CASE
Beggiato E, Freilone R, Anselmetti GC, Tartaglino B
*Ematologia-Oncologia ASL 6 Ciriè Torino; *Radiologia Istituto per la Ricerca e la Cura del Cancro IRCCS Candiolo, Torino, Italy

Introduction: Patients affected by multiple myeloma (MM) who present a relapse after high doses chemotherapy have little therapeutic options. Often in advanced stages of the disease patients present progression of osteolytic lesion, bone pain and fractures. Thalidomide is currently used as promising therapy in MM thanks to its antiangiogenetic and immunomodulatory properties. Percutaneous vertebroplasty with acrylic cement (polymethylmethacrylate, PMMA) consists of injecting PMMA into the vertebral bodies weakened by osseus lesions. It is a minimally invasive procedure that is effective in the treatment of painful osteolytic vertebral disease. Case report: In June 1999 a 63 years old man was referred for widespread bone pain. A skelatal X-ray showed diffuse osteolytic lesions in the dorsal and lumbar section of the spinal column with pathological tissue at L5 level. Multiple myeloma was diagnosed with IgG lambda protein (IgG 7806 mg/dL, marrow examination showed 70% plasma cells, Bence Jones proteinuria was absent. The patient underwent chemotherapy with six DAV courses achieving a complete remission. A maintenance regimen was started with dexamethasone and pamidronate. Nine months after the end of chemotherapy the patients relapsed and underwent a double autologous peripheral blood transplantation (with melphalan 100 mL/m2 as conditioning regimen) followed by maintenance therapy with interferon alpha and dexametasona. After one year (June 2002) the patient accused intense back pain, MRI showed a progression of the osteolytic lesions in the lumbar section of the spinal column with pathologic tissue at T11 and initial spinal cord compression; the IgG were 2290 mg/dL and bone marrow plasma cells 5%. The patients underwent local radiotherapy (RT) at T11 and steroid high dose therapy that was soon interrupted for the appearance of Herpes Zoster lesions on the chest. A morphine sulphate therapy (30 mg day) was begun for pain reduction. At completion of RT and resolution of Herpes Zoster the patient showed a progression of monoclonal component (IgG 6620 mg/dL) and started a therapy with thalidomide 100 mg/day.
continuously and dexamethasone 40 mg day 1-4 every month. After three months IgG were 2926 mg/dL but the patient continued to assume morphine sulphate for persistent back pain. Following a MRI a vertebroplasty was then performed on L3 and L5 the principal points of bone loss and pain. Immediately the patient experienced no further pain and ended morphine therapy. At present the patient is continuing the therapy with thalidomide and steroid with stable IgG values <3000 mg/dL. Conclusions. Thalidomide is an effective therapy for refractory-relapsed M M. In this case the association of thalidomide and vertebroplasty allowed for a stabilization of the disease without necessity of pharmaceutical pain therapy and hence for a significant improvement in the quality of life of the patient.

PU146
THE ADDITION OF ORAL MELPHALAN IMPROVES RESPONSE TO THALIDOMIDE IN PATIENTS WITH ADVANCED MULTIPLE MYELOMA: A CASE-CONTROL STUDY
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So far multiple myeloma (MM) is an incurable disease because of its inherent resistance to chemotherapy. Therefore, new agents are needed to treat this troublesome disease. Many phase II studies demonstrated thalidomide has significant activity in patients with de novo or relapsed/refractory MM. The results seems to improve adding dexamethasone or chemotherapy but no comparative studies were published. We report our experience using a combination of thalidomide and oral melphalan compared with thalidomide alone in a case-control study including patients with advanced MM. From May 2000 to July 2002, 27 patients were treated with thalidomide plus oral melphalan (TM group) and 23 patients with thalidomide alone (T group). Patients were not excluded because of either poor performance status or cardiopulmonary, renal and liver disfunctions. The initial dose of thalidomide was planned to be 100 mg p.o. daily at bedtime, escalated weekly by 100 mg increments until a maximum dose of 600 mg daily continuously until side effects or disease progression were documented. Melphalan was administered intermittently at a dose of 0.20 mg/kg/d orally for four days every 28 days for almost one course after greatest response or until severe toxicity were assessed. No patients received antithrombotic prophylaxis. Prognostic features such as β2-microglobulin, hemoglobin level, prior regimens, prior high-dose therapy and disease history did not significantly differ between the two groups of patients while age was significantly lower in the TM group (69 vs 74 years; p=0.042) and median follow-up was significantly longer in the TM group (13 vs 10 months; p=0.022). Rate of paraprotein decrease ≥ 50% was significantly higher in the TM group compared with T group (63% vs 26%; p = 0.009). Remarkably, ≥ 75% paraprotein decrease was obtained in 4 patients (15%; true CR) of TM patients compared with only 1 (4%; no true CR) of T group. The median time to remission was significantly shorter in the TM group (6 weeks vs 10 weeks; p= 0.0312). Multivariate analysis selected only TM therapy (p = 0.008) as factor associated with better response. After a median follow-up of 13 months (range 6-32), 18 patients (36%) had disease progression and 11 (22%) died. Eight patients died of disease progression, and 1 patient died for pulmonary embolism, infection and heart failure, respectively. PFS was significantly longer in TM group compared with T group (median NR vs 13 months; 61% vs 45% at 2 years; p=0.0356) whereas OS did not, most likely because of a median follow-up significantly shorter in the T group. In the multivariate analysis only response ≥ 50% was associated with higher PFS at two years (66% vs 42%; p=0.0464) and only Hb ≥ 10.5 mg/dL significantly affected longer OS (76% vs 49% at 2 years; p=0.0449). Fourteen patients (28%) stopped and 24 (48%) reduced thalidomide because of side effects. In the TM group, 4 patients (15%) delayed the administration of melphalan because of leukopenia. The main side effects attributable to thalidomide were constipation (72%), somnolence (38%), asthenia (24%) and sensory peripheral neuropathy (44%). Central nervous system adverse effects (dizziness, headache) were found in 8 patients (16%) although severe toxicity was rare. No differences were found between the two groups with respect to the above side effects. Although not statistically significant, rate of deep venous thrombosis (11% vs 4%; p=0.614) and grade 3 (WHO) leukopenia (30% vs 13%; p=0.073) were higher in the TM group without severe infections increase. This study suggests that oral melphalan added to thalidomide improves response rate and PFS in advanced poor prognosis multiple myeloma without increasing severe toxicity. Consequently, the combination of thalidomide plus oral melphalan should be further investigated in the context of randomized studies.
AL amyloidosis is a relatively rare disorder resulting from the deposition of immunoglobulin light-chain insoluble fragments within organs and tissues. The disease is diagnosed by biopsy evidence of amyloid deposition in affected organs or by subcutaneous abdominal fat aspiration or rectal biopsy. The diagnosis is confirmed by the presence of serum or urine monoclonal light chains and/or clonal plasma cells in the bone marrow. The disease course is characterized by progressive organ impairment and, without treatment, the majority of AL patients die within two years of diagnosis. Several reports have shown that inhibition of amyloidogenic protein production improves amyloidosis. This finding and the poor prognosis of AL amyloidosis has led to an increasingly frequent use of intensive treatment regimens similar to those used in myeloma. Currently, high-dose chemotherapy followed by autologous PBSC in selected patients seems to be the most effective treatment. The major drawback of this therapeutic approach is that transplantation-related mortality (TRM) in AL amyloidosis patients is 4 to 8 times higher than that in patients with multiple myeloma and remissions are often short lasting. Experience gained has shown that the only potential cure for myeloma is represented by allo-SCT, but unfortunately this procedure is associated with a high mortality risk. Recent reports on myeloma have demonstrated that tandem auto-allo transplants with non-myeloablative conditioning protocols provide rapid and sustained engraftment with durable and complete donor chimerism. Since this highly effective therapeutic approach is associated with tolerable toxicity, low treatment-related mortality and a high rate of complete remission, we decided to evaluate its feasibility in AL amyloidosis. A 53-year-old man with a diagnosis of nephrotic syndrome and AL amyloidosis presented to our Center in August 2002. On admission, the patient had proteinuria (38 g/24h), ventricular septal thickness of 17 mm, and high serum cholesterol levels. Bone marrow biopsy revealed marked amyloid infiltrates and 5-10% of clonal plasma cells. Involvement of other organs was excluded. After obtaining the patient’s written informed consent, PBSC were mobilized alone with Filgrastim (5 μg/kg of body weight, twice daily), collected during 2 consecutive leukaphereses and cryopreserved. In November 2002, following a conditioning regimen with Melphalan 100 mg/m², the patient received 5.5×10⁶/kg of autologous CD34+ cells. The auto-transplantation procedures were well tolerated and no serious adverse events were observed. In March 2003, he underwent allo-SCT from an HLA-identical sibling. Conditioning comprised melphalan 80 mg/m² (day -6), fludarabine 25 mg/m² (days -5, -4, -3, -2) and ATG 5mg/kg (days -3, -2). GVHD prophylaxis was carried out with Cyclosporine and Methotrexate. The post-transplant course was uneventful and the patient was discharged from the hospital on day +28 with complete donor chimerism. During the months of follow-up 24/h proteinuria continued to decrease and on recent examination was 11.6 g. Although follow-up in our patient is not yet sufficient to evaluate the efficacy of this two-phase approach on amyloid deposits and clinical remission, the results do seem to suggest that auto-SCT + allo-SCT with non-myeloablative conditioning protocols is feasible for selected patients with AL amyloidosis. However, larger series of patients are required to assess the value of this treatment strategy.

Thalidomide, a sedative drug obsolete because of its teratogenicity, has proven to be effective in multiple myeloma (MM) patients, although the mechanism is still unclear. After the first study, carried out by Barlogie’s group in 1999, several studies have shown the effectiveness of thalidomide in relapsed and refractory patients, with response rates varying up to 64%. The efficacy of thalidomide appear to be dependent by initiation of angiogenesis, as well as by decrease of adhesion molecule ICAM-1 expression on plasma cells, block of IL-6 TNF and IL-1 effects, enhancement of TNF-γ and IL-2 production. From May 2001 to June 2003, we treated with thalidomide 13 patients with relapsed or refractory MM. The patients included 5 men and 8 women with a median age of 77 years (range: 54-80 years). All patients had advanced disease (stage III). Two patient had previously undergone high dose therapy. Prior standard therapy included MP, VAD, CTX+PDN, VBM CP, Desametasone. Two patients had undergone one previous line of therapy, five three lines, three four lines, two five lines. The paraprotein was IgA-κ (3 patients), IgA-λ (2 patients), IgG-κ (5 patients) and IgG-λ (3 patients). The doses of thalidomide used ranged from 100 to 600 mg/day. Thalidomide as a sin-
A single agent was prescribed for 7 patients; in five patients, it was administered in combination with Desametasone (16-40 mg/day × 4 day/month). After a median follow-up of 8.6 months (range 1-25), 4 patients died 3, 3, 13 and 21 months after the first administration of thalidomide. Two patients are not evaluable for response because their follow-up is still too short. Of 11 evaluable cases, a complete response was achieved in two patients, a partial response in six, with a response rate of 73%. One patient has a stable disease. One did not respond and died of progressive disease while on therapy. The most frequent adverse effects were constipation (50%), paresthesias (50%), sedation (40%), and, less frequently, tremor (20%), hyposthenia (20%), edema, dyspnea, bronchospasm (10%), dizziness (10%). Reduction of dose, if needed, usually allowed the continuation of treatment. In one case, leg vein thrombosis was documented. Moreover, we registered a relatively elevated incidence of infections (55%). Particularly, two patients in CR and PR respectively, died at 13 and 21 month after first thalidomide administration because of bacterial encephalitis and severe bronchopneumonitis. Altogether, a good response has been obtained in this setting of patients. However, we believe that the occurrence of uncommon infectious events, even in condition of responding disease and clinical remission, should induce consideration of the possible pro-infective role of the drug, probably related to its immunosuppressive activity, especially in older patients. This consideration suggests the appropriateness of antinfective prophylaxis during treatment.

**PU149**

**BONE MARROW ASPIRATE SHOWING CONGOPHILIC DEPOSITS IN SYSTEMIC AMYLOIDOSIS: A CASE REPORT**

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Primary amyloidosis (AL) is a plasma cell disorder in which neoplastic clonal plasma cells in the bone marrow produce monoclonal immunoglobulin light chains that form protease-resistant amyloid fibrils. AL fibrils accumulate within tissues systemically, causing progressive multiorgan impairment. The most commonly involved organs in AL are the kidneys, the heart, the soft tissues and the peripheral nerves, although the amyloid deposition may occurs everywhere and can be documented in the bone marrow (BM) by trephine biopsy. We report on the case of a 68 woman with AL who was referred to our attention for a clinical re-assessment on December 2002. She was previously admitted in an other Centre because of malaise and weight loss, one year before. The patient underwent to several labora-
SYMPTOMATIC REFRACTORY MULTIPLE MYELOMA
ZOLEDRONIC ACID IS AN EFFECTIVE OPTION FOR ELDERLY

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Background. Osteolytic bone destruction is the hallmark of myeloma. IL-6 plays a pivotal role both in the growth and survival of myeloma cells, blocking their apoptotic pathway. Striking, IL-6 secretion blocks the death of myeloma cells induced by corticosteroids but has little, if any, effect on tumor cell death triggered by radiation. Moreover, a reduction in the production of the cytokine IL-6 from myeloma bone marrow stromal cells exposed to bisphosphonates has also been found. Zoledronic acid is the most recent addition to the clinically available intravenous (IV) bisphosphonates. Clinical benefit includes improvement in bone pain, reductions in skeletal events and delay in time-to-first-skeletal-events. Samarium-153-EDTMP is a 1:1 complex of radioactive Samarium-153 and a tetraphosphonate [ethylenediamine-tetramethylene phosphonic acid (EDTMP)]. 153Sm-EDTMP has a high affinity for skeletal tissue and concentrates by chemiabsorption in areas of enhanced metabolic activity, where it associates with the hydroxyapatite crystal. Despite the efficacy of 153Sm-EDTMP as a palliative agent for the treatment of bone metastases is well-known, its effects in patients with symptomatic refractory multiple myeloma (MM) have not been documented. Nevertheless, the synergistic effects of combining radioactive and non-radioactive bisphosphonate is not known. We investigate toxicity, clinical impact and quality of life (QoL) of a sequential dose of 153Sm-EDTMP and zoledronic acid in 8 symptomatic refractory MM patients. Materials and methods. Inclusion criteria: refractory MM diagnosis on pain control, normal renal function. Median age was 79 years (range 71-81 yrs) M:F=6:2; ECOG PS >= 2; median time from diagnosis to targeted therapy = 2 years (range 1-3 yrs); 4 patients presented with IgG kappa, M-component. All patients were in DS stage IIIA and 3 of them had received >2 previous chemotherapeutic treatments. Two GBq of 153Sm-EDTMP was scheduled to be administered every 12 weeks and 4 mg of zoledronic acid every 28 days. At the time of enrollment all 8 patients complained of severe, persistent pain, requiring a combination of nonopioid analgesics. Patients were asked to quantify their pain using a visual analog scale which was then converted into a numeric pain-rating scale (range 0-10). At baseline, the median pain-rate was 7 (range 7-9). QoL assessment was based on Therapy Impact Questionnaire. Results. In 5 out of 6 patients, two courses of 153Sm-EDTMP plus zoledronic acid were sufficient to produce long term improvement in MM-related symptoms. Two patients received one course and are too early to evaluate. A third additional cycle of therapy was necessary only for one patient. After treatment the median pain rate was reduced to 2 (range 0-3), compared to 7 (range 7-9), with a median follow-up time of 10 months (range 8-13 months) with no need for analgesics. No hematological or extra-hematologic side effects were observed. Interestingly, M-component decreased more than 25% in 3/6 patients and at last FU was still stable with no evidence of increase. Discussion. Our experience, although based on a limited number of patients, provides a novel palliative approach to the treatment of symptomatic elderly MM patients not eligible for further chemotherapy.

PUI51 EOSINOPHILIA IN MYELOMA PATIENTS TREATED WITH
THALIDOMIDE, DEXAMETHASONE AND CYCLOPHOSPHAMIDE
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Treatment with thalidomide, alone or in combination, is one of the major improvements in the recent treatment of multiple myeloma. Among side effects, modifications of hematologic parameters have been reported and, in some patients, an increase in eosinophil count has been observed in conjunction with other side effects induced by thalidomide. On this basis we retrospectively examined medical records of 39 patients affected by resistant or refractory multiple myeloma treated with a combination of thalidomide 200 mg, cyclophosphamide 100 mg, both continuous-ly, and dexamethasone 40 mg for 4 days every month. Before starting therapy, median eosinophil count had increased to 109/L and it increased to 209/L after the first month, 1309/L after the second month, and 1209/L after the third month. M-component decreased more than 25%ing entire duration of treatment was over baseline (pre-

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therapy we observed that the median eosinophil count during treatment was $0.26 \times 10^9/L$ vs $1.12 \times 10^9/L$ of responding patients. In addition, none of the common thalidomide’s side effects was correlated to the eosinophilia. In conclusion, we found that in myeloma patients treated with a combination of thalidomide, cyclophosphamide and desamethasone, increase of eosinophil count was a very common event. This finding cannot be ascribed to desamethasone because actually it may induce eosinopenia. On the contrary, the combination of thalidomide and cyclophosphamide may be responsible for eosinophilia and the increase of eosinophil count could be related to thalidomide’s mechanism of action. In fact, in non-responding patients the eosinophil count was lower than in responsive ones but the number of the former patients is too small for differences to be significant. Evaluation of circulating cytokines during thalidomide treatment could help in explaining this phenomenon.

**PU152**

**IGM MYELOMA: A RARE SUBTYPE. A DESCRIPTION OF 4 CASES**

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The distinction between multiple myeloma (MM) and Waldenström’s macroglobulinemia (WM) usually poses no diagnostic dilemma. Consistent with a diagnosis of MM is the presence of an IgM monoclonal gammopathy associated to multiple osteolytic lesions and plasma cell infiltration of the bone marrow. On the other hand, characteristic of WM is the presence of IgM monoclonal gammopathy associated to lymphadenopathy, hepatosplenomegaly, anemia, and hyperviscosity syndrome in conjunction with a monoclonal lymphoplasmacytoid proliferation in both the bone marrow and peripheral blood. Despite that, few cases of IgM myeloma have been reported, with clinicopathologic features intermediate to those of MM and WM. We present 4 patients with an IgM monoclonal gammopathy in whom morphologic and clinical features were consistent with the diagnosis of IgM myeloma. From July 1973 to April 2002, we observed 3,176 monoclonal gammopathies of which 316 (9.9%) were of IgM type. At diagnosis, 187 (59.1%) were MGUS, 93 (29.4%) were WM, 10 (3.1%) were non Hodgkin’s lymphoma (NHL) and only 4 (1.3%) were IgM Myeloma. Of the 186 MGUS, 21 (11%) evolved to WM and 1 to NHL during the follow-up. The clinical characteristics of the 4 IgM myeloma are reported in Table 1. None of the 4 patients had diffuse osteolytic lesions. However, in case 2, who complained lumbar pain and paresthesia, magnetic resonance demonstrated the presence of L2-L5 fractures with associated pathologic tissue causing spine compression. The histological analysis of this tissue showed a diffuse plasma cells infiltration. After laminectomy, the patient received local radiotherapy obtaining a reduction of symptoms but few months later he died of progressive disease. Of the remaining 3 patients only case 3 who had a smouldering IgM myeloma is still alive after 172 months from diagnosis. In conclusion, IgM myeloma is a rare disease, accounting for about 1% of all monoclonal IgM and less then 0.5% of MM. The distinction between the WM and MM rests on the histological finding of a lymphoplasmacytoid proliferation in WM as opposed to the predominantly plasma cell rich infiltrate in myeloma. Since MM and WM differ in prognosis and treatment strategies, the two disease entities should be distinguished based on clinical criteria and bone marrow morphology.

**PU153**

**CORRELATION BETWEEN HEMOGLOBIN AND FATIGUE IN MULTIPLE MYELOMA PATIENTS**

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Fatigue occurs commonly in cancer patients and negatively affects their quality of life (QOL). The aim of this cross-sectional study was to evaluate the relationship between fatigue, using the FACT-An questionnaire, and hemoglobin (Hb) level, and other patient characteristics in multiple myeloma (MM) patients. The study included 1071 adult patients affected by MM regard-

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SD: stable disease; PD: progressive disease; MP: melphalan+prednisone; RT: radiotherapy.
less of disease or treatment status. FACT-An scores were directly related to Hb level, increasing from 44.2 in patients with Hb < 9 g/dL to 60.3 in those with Hb > 14 g/dL. The relationship between Hb levels and FACT-An scores was confirmed when examined within a multiple logistic regression model adjusting for the effect of several covariates. Although QOL was significantly affected by patients' characteristics such as age, sex, or tumor status, the regression slope of FACT-An scores on Hb levels was reduced only to a limited extent compared with the univariate regression. There were no significant interactions between Hb and the other model factors. This study provides strong, if indirect, evidence that correction of anemia should be tried to improve the QOL of MM patients. Moreover, the results of this study suggest that patients' QOL may benefit from correction of anemia until sex-specific normal Hb values are attained.

PU154
RARE SEVERE HYPERGAMMAGLOBULINEMIA IN VISCERAL LEISHMANIASIS
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Visceral leishmaniasis is a worldwide, disseminated intracellular protozoal infection that usually manifests by fever, hepatosplenomegaly, anemia, thrombocytopenia, leukopenia and hypergammaglobulinemia. Here we report the case of a 49 years old obese (kg 150, h 160 cm) man with pancitopenia (HB 8.4gr /dL, WBC 2400 mm3, PLT 99000 mm3) and a very important hypergammoglobulinemia (tot protein: 13.6 gr /dL, γ: 12.84 mg/L, λ: 11.35 g/dL). We begin a differential diagnostics for hypergammoglobulinemia (tot protein: 13.6 gr /dL, γ: 12.84 mg/L, λ: 11.35 g/dL), protein C reactive level (4,36 mg/dL), VES 132, PCR 43.2 mg/l, creatine 1.7 mg/dL, CA 19-9 1482 U/mL, Leishmania antibodies neg, DÁT pos, no osteolitic lesion, TC total body: hepatosplenomegaly. Bone marrow aspirate and biopsy: presence of Leishman -Donovan bodies in hypercellular marrow. Although hypergammaglobulinemia is a usual finding, this very important hypergammaglobulinemia has rarely been reported in association with this disease.

PU155
PERITONEAL EFFUSION AS SIGN OF PROGRESSION IN LIGHT CHAIN MYELOMA
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About 20% of patients with plasma cell myeloma have monoclonal immunoglobulin light chain. In these patients, as well as in all cases of multiple myeloma, extramedullary disease manifestation is observed with increasing frequency. Although uncommon at diagnosis, with the extension of the overall survival, we can detect a plasma cell involvement in several organs. We report a case of k light chain Myeloma in a 75-years-old man, Stage IIIA at onset, for vertebral pathological fracture and secondary medullar compression, with unusual localisation at progression time. He was treated with polichemioterapy VCAP scheme (with vincristine, cyclofosfamide, farmorubicine, and prednisone) for 7 cycles, with partial response, and than with VCPM (with vincristine, cyclofosfamide, melphalan and prednisone) for 6 cycles, with stable disease. After 3 months of last chemotherapy administration, and after 1 year from diagnosis, the patient started to have abdominal pain. By ultrasonografy (US) investigation we found a peritoneal effusion, with collecting of 1500ml in the first paracentesi procedure and 2000ml in the second one. By cytological study of both collections we found a peritoneal plasmacellular invasion. The morphological characteristics of these cells were of immaturity, with expression of CD 138 and CD20. By US investigation of abdominal organs we didn’t find focal lesions or pathological lymph nodes. The patient was newly treated with VCPM scheme, with no more peritoneal effusion after 1 cycle. At time of peritoneal invasion the disease restaging showed a stable bone marrow plasmacellular infiltrate (about 30% of total cellularity), no new osteolytic lesions, and stable Bence Jones proteinuria. Progression signs were β2microglobulin level (12,84 mg/L), protein C reactive level (4,36 mg/dL), VES (59 mm/h). Some authors described pleural localisation in course of Multiple Myeloma, while peritoneal invasion results to be extremely rare. We observed a case of light chain myeloma with progression in unusual organ as peritoneum, without progression in usual target organ. Clinical response seems to be good, after only one cycle of chemotherapy.
Waldenstrom’s disease is a B-cell malignancy with abnormal production of a monoclonal IgM protein. Generally the bone marrow biopsy is diffusely infiltrated with lymphocytes, plasmacytoid lymphocyte and some plasma cells. It is controversial whether HCV is involved in B-cell lymphoproliferative disorders, also in cases of macroglomulinemia, but some authors have associated the HCV infection with development of Waldenstrom’s macroglobulinemia, after resolution of macroglomulinemia with interferon α treatment. We observed a 66-year-old man with an asymptomatic IgM k monoclonal component (MC) associated the HCV infection with development of Waldenstrom’s disease, after resolution of macroglomulinemia with interferon α treatment. We started to treat the patient with combined antiviral therapy: PEG-interferon and Ribavirin. After 4 month with this treatment the MC is decreased from initial level of 5.4 g/dL to 0.7 g/dL, despite a reduction dose due to hematologic toxicity. These case show a strict association between HCV infection and Waldenstrom’s disease, with a very particular manifestation, without bone marrow localization.

Thalidomide have emerged as effective drug in patients affected by multiple myeloma (MM), either in advanced or in primary disease. We report a 3-year experience of four hematologic institutions in patients affected by relapsed or refractory multiple myeloma treated with thalidomide alone or in combination with dexamethasone (Dexa). Thalidomide was subministered at initial standard dose of 100 mg/d with a dose escalation every two weeks until a maximum dosage of 800 mg/d or to the maximum tolerated dosage. We enrolled from February 2000 to October 2001, 40 pts, with age ranging from 49 to 86 years (median 69). Among 40 pts., in 25 M- component isotype was IgG, 9 IgA, light chains in 4, 2 were affected by plasma cell leukemia. All of them had received at least one line of prior chemotherapy; 3 patients were previously treated with autologous stem cell transplantation. In 25 pts (62.5%) treatment with monthly Dexa at standard dose was added, and in 35/40 monthly diphosphonates were subministered. Among 40 pts, 18 (45%) obtained a response: 2 pts a complete response (no M.-Component at immunofixation), 6 a Major Response (MC reduction > 75%), 6 a Partial Response (MC reduction > 50% < 75%), 4 a minor Response (MC reduction < 50% > 25%). Among the 22 pts who failed treatment, 4 were forced to stop thalidomide early because of side effects, consisting in fever (1), somnolence (1), erithema (1) and cramps (1). No patient reached maximum planned dose of 800 mg, median daily dose was 200 mg. Concerning overall toxicity, it was mainly represented by neurologic symptoms, such as tremor, constipation, parasthesias and somnolence. These symptoms were often dose-limiting. Two pts (5%) presented deep venous thrombosis. Out of 18 responders, 9 (50%) relapsed; median time to relapse was 10 mos (range 2-36 mos). Four pts died in remission because of myocardial infarction (1), blood transfusion refusal (1), uncertain sudden death (2); 4 pts are alive in remission from 18 to 39 mos. (median 32 mos.). Overall median survival is 10 months (range 1 - 39+ mos.).
High serum levels of sMUC-1 have been shown in patients with adenocarcinoma and multiple myeloma (MM). It has been shown that patients with multiple myeloma (MM) have high peripheral blood and bone marrow levels, and that the latter directly correlate with tumour mass. To define the prevalence of high sMUC-1 levels in patients with plasma cell dyscrasias, we tested 89 monoclonal gammopathy of undetermined significance (M GUS), 76 MM and six plasma cell leukemia (PCL), admitted consecutively to our Institution during the last ten years. Peripheral blood samples were collected at the time of diagnosis or at any time during follow-up from M GUS patients; for MM and PCL patients we analyzed stored serum aliquots, collected at the time of diagnosis. Samples obtained from 65, age and sex matched, healthy subjects, attending our Transfusion Department, were also evaluated. All of the samples were tested using Immunolite B27.29 antibody against MUC-1 protein. The sera from the MM/PCL patients were also tested using Abbot IMX CA15.3, Boehringer Mannheim Enzymmun CA15-3 and Centocor CA15-3 in accordance with the manufacturers’ protocols. High sMUC-1 levels were found in 11/89 subjects with M GUS (12.4%), 13/76 with MM (17.1%) and 3/6 with PCL, while in the healthy control group only one subject had high levels (1.5%) (p=0.001). The mean sMUC-1 levels were significantly higher in the monoclonal Component carrying patients than in our healthy subjects (43.2 vs 26 U/mL; p=0.001). The median follow-up of the 82 MM/PCL cases was 30 months (range 6 -114), during which 51 patients died: 39/66 (59%) with normal and 12/16 (75%) with high sMUC-1 levels. The median overall survival (OS) was 44 months. There was a difference in OS between the MM/PCL cases, had increased sMUC-1 levels. The frequent presence of high MUC-1 levels in PCL patients suggest a possible relationship between them and tumor malignancy (cell proliferation, genetic instability). In patients with MM, high sMUC-1 levels identify a group with a poor prognosis, showing a possible role of MUC-1 in tumor progression. Given the short follow-up of the M GUS patients (median 12 months; range 6 -30) and the absence of any MM transformation during this time, no conclusions can be drawn concerning a possible correlation between high sMUC levels and the relative risk of M M evolution.
and joint pain. After two plasma-exchange, in May 2002 we started a treatment with four week administrations of rituximab 375 mg/m², followed by two subsequent administrations every 15 days, for a total of six administrations. The association with pegylated IFN at 80 µg/week was maintained. A rapid improvement of leg pain and skin ulcers was seen after rituximab treatment. Both rituximab and pegylated IFN were interrupted in August 2002. An increase of HCV copies from 92.000 to 589.000 after rituximab therapy was observed, but it was associated neither with ALT increase nor other liver function failure signs. She is now well ten months after the end of any treatment. Conclusions. In this severe HCV-related MC, resistant to other conventional treatment strategies, the association of rituximab and interferon was safe and highly effective. Prospective cooperative trials of rituximab associated to interferon was confirmed by immunofixation as IgGk. Laboratory studies detected ANA (1:2360) and RnP (102 E.U./mL). There were not clinical and laboratory criteria for multiple myeloma's diagnosis. A mixed connective tissue disease was diagnosed. Chemotherapy with melphalan and prednisone was started. After chemotherapy the clinic examination demonstrated a significant improvement and a reduction of antibody anti-RnP (78 E.U./mL) was observed. The patient was lost to follow up. Conclusions. Numerous skin disorders are associated with monoclonal gammopathy. They can be differentiated in the following way: 1) specific skin lesions caused by monoclonal immunoglobulin producing cells in the skin (MM, M W); 2) functional disturbances induced by monoclonal immunoglobulins if they are cryoglobulins (Raynaud syndrome), or by interaction between monoclonal component and lipoproteins (xanthomatosi); 3) skin diseases associated with monoclonal gammopathies where the role of monoclonal component, or cytokines, or other unidentified factors released from the malignant plasma cells is unknown (pyoderma gangrenosum, acrodermatitis atrophicans, and psoriasis). Our cases are included in the last group because it is not possible to emphasize the role of the immunoglobulins in the pathogenesis of skin disorders.

### References

Peripheral neuropathy is one of the major side effects of thalidomide (thal) in MM patients: objectives of this study is to evaluate thal-induced neuropathy using electrophysiological studies. We prospectively performed longitudinal neurologic examination and nerve conduction studies (NCS) before and during thal treatment in 9 patients affected by relapsed or refractory MM in an open-label trial of thal. NCS included recording of sensory nerve action potentials (SNAPs) from median, radial, ulnar, and sural nerves. SNAP amplitudes for each nerve were expressed as the percentage of its baseline, and the mean of the four was termed the SNAP index. A 40% decline was considered significant. F-waves were obtained from the median and peroneal nerves and F-waves chronodispersion was studied. 9 patients remained on thal at 3 mos., 5 remained at 6, 9, 12 mos., 4 remained at 15 mos., and 1 remained at 18 mos. 2 patients discontinued thal after 3 and 12 mos. respectively because of progression of MM. 2 patients are on thal from 3 mos and are on follow-up. None of the patients at 3-mos. evaluation and at 6-mos. evaluation developed neuropathy. All 5 patients evaluated at 6 months showed abnormal F-waves chronodispersion. All 5 patients evaluated at 9 months showed a sensory more than motor, axonal polyneuropathy that presented as paresthesias or numbness. The average cumulative dose of thalidomide received was 38.4 gr. (30-45 gr.). After the detection of neuropathy thalidomide was continued in 2 patients at lower dosage (100 mg/day) and in 2 patients for brief monthly courses (100 mg/day for 10 days a month), with mild worsening of electrophysiological findings. Clinical symptoms and a decline in the SNAP index occurred concurrently. F-wave chronodispersion was found prior to changes in SNAP amplitudes. These results suggest that a close clinical and electrophysiologic follow-up while on treatment is recommended; F-chronodispersion will help in early detection of neuropathy, while the SNAP index can be used to monitor neuropathy.
TECHNETIUM-99M SESTAMIBI SCINTIGRAPHY IN THE DETECTION OF MYELOMA BONE DISEASE

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Technetium-99m 2-methoxy-isobutyl-isonitrile - (99m)Tc-sestamibi - has been proposed as a potential tracer in patients with multiple myeloma (MM), as its increased uptake in the bone marrow has been reported as indicator of myeloma activity. The aim of this study was to explore the role of (99m)Tc-sestamibi scintigraphy in the detection of bone marrow involvement in patients (pts) with multiple myeloma (MM) and to assess the relationship of such scintigraphic pattern of the disease in the follow-up of pts with M M or monoclonal gammopathy of undetermined significance (M GUS).

Methods: between January to March 2003 twelve consecutive pts were enrolled in this study. Pts characteristics are summarized in Table 1.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Disease status</th>
<th>99mTc-MIBI score</th>
<th>X-ray skeletal survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>M</td>
<td>MM - IgG/K</td>
<td>Partial remission after CHT</td>
<td>Pos *</td>
<td>Neg</td>
</tr>
<tr>
<td>56</td>
<td>F</td>
<td>MM - IgG/L</td>
<td>Progressive disease</td>
<td>Pos ++</td>
<td>Pos</td>
</tr>
<tr>
<td>61</td>
<td>F</td>
<td>MM - IgA/L</td>
<td>Partial remission after ABMT</td>
<td>Pos +</td>
<td>Neg</td>
</tr>
<tr>
<td>70</td>
<td>F</td>
<td>MGUS</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>71</td>
<td>F</td>
<td>MM - IgG/L</td>
<td>Partial remission after CHT</td>
<td>Pos +</td>
<td>Pos</td>
</tr>
<tr>
<td>63</td>
<td>F</td>
<td>MM - IgA/K</td>
<td>Complete remission after ABMT</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>69</td>
<td>N</td>
<td>MM - IgG/K</td>
<td>Progressive disease</td>
<td>Pos ++</td>
<td>Pos</td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td>MGUS</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>66</td>
<td>F</td>
<td>MM - micro</td>
<td>Partial remission after CHT</td>
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<td>Progressive disease after ABMT</td>
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<tr>
<td>62</td>
<td>F</td>
<td>MM - IgG/L</td>
<td>Progressive disease</td>
<td>Pos +++</td>
<td>Pos</td>
</tr>
</tbody>
</table>

Five out of F12 MM pts had active disease and 5/12 were in complete or partial remission after conventional or high dose chemotherapy. The semiquantitative score (99m)Tc-sestamibi uptake was positively correlated with the percent of myeloma cells infiltrating the bone marrow and the biochemical indicators of disease activity. Results: all MGUS and only one MM pts (complete remission after autologous BM T) showed a negative (99m)Tc-sestamibi scan. Among the 9 MM pts showed a positive scan, a different uptake score was observed. It is correlated significantly with the disease status and all the most relevant clinical variables. Comparison with X-ray skeletal survey showed discordant results in 3 pts (3 negative X-ray survey with positive sestamibi scans). Conclusions: these results confirm that whole-body (99m)Tc-sestamibi scintigraphy is a sensitive imaging technique which reliably reflect myeloma disease activity in bone marrow.

TC99M-SESTAMIBI IN FOLLOW UP OF SOLITARY PLASMACYTOMA

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Tc99m-sestaMIBI has been found to concentrate in some neoplastic tissues and recently in multiple myeloma (M M). This latter issue is at present under evaluation; the results acquired so far seem to suggest this tracer high sensitivity and specificity in diagnosing and in following up MM. Solitary plasmacytoma (SP) is a clinical variant of neoplastic plasmacell proliferation, localized in bone (SBP) or in soft tissues mainly of the upper respiratory tract (extramedullary plasmacytoma - EP). Radiotherapy and surgery are the most adopted treatments allowing significant possibilities of cure: however, the disease progresses in about 50% of patients affected by SBP and in 8-30% of patients affected by EP. This possibility opens the way to adjuvant chemotherapy. We are studying the usefulness of Tc99m-sestaMIBI scintigraphy in the follow-up of patients affected by SP. From June 1997 to December 2002 we have followed eight patients affected by SBP or EP. In all cases the diagnosis was based upon histology and exclusion of multiple myeloma. Patients were treated with local procedures (radiotherapy, surgery), often followed by chemotherapy and, in some cases, by auBMT. After treatment, patients were reassessed by standard procedures to define disease activity, including variations of local signs and symptoms evaluable by physical examination, by CT scans or MRI, variations of biochemical parameters and in one case of local histology. Tc99m-sestaMIBI scintigraphy was repeated during the follow-up and correlations between standard procedures and scintigraphy were evaluated by the Chi-square test. In 7/8 patients there was full concordance: tracer uptake was always normal in remission and focal uptake was demonstrated in two
relapsed patients. One patient had normal uptake after treatment, but MRI suggested persistence of neoplastic tissue inside a symptomatic collapsed vertebral soma. The patient remained in stable condition without further therapy for 20 months, when signs of progression appeared (progressive increase of serum MC) and focal tracer uptake became again visible. In this small group of patients sensitivity of Tc-99m MIBI scintigraphy was 85%; no false positive case was diagnosed. However, as normal uptake after treatment cannot exclude false negative cases at diagnosis, we evaluated also scans available at diagnosis in 6/8 patients. The tracer detected focal lesions in 5/6 patients: one patient was considered false negative, because he had normal uptake, but histology of the costal tumor showed plasma cell infiltration. Tc-99m MIBI scintigraphy presents some advantages in comparison with other imaging methods (X-ray, CT, MRI): the possibility to identify solitary lesions in bone or in soft tissues and to evaluate disease extension in a single scan and the possibility to discriminate between residual active or inactive lesions. We believe that basal Tc-99m MIBI scintigraphy is advisable to exclude possible false negative during the follow up.

PU165
TC99M-SESTAMIBI UPTAKE IN NON SECRETORY MULTIPLE MYELOMA
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Tc99m-sestaMIBI is emerging as a tracer of neoplastic plasmacytic proliferation, with sensitivity and specificity of about 80%. As it concentrates inside plasma cell cytoplasm, it can detect both diffuse bone marrow uptake and focal uptake in bone or soft tissues. Extension and intensity of diffuse uptake can be semi-quantitatively measured and connected to the percentage of bone marrow plasmacell infiltration. Starting from these premises, we tried to evaluate the information deriving from Tc99m-sestaMIBI in particular subsets of patients who cannot be easily diagnosed and followed up by standard procedures. This is the case of nonsecretory or scanty secretory multiple myeloma (nSM), which accounts for less than 5% of all cases of MM, in which the diagnosis may be delayed because of lack of recognizable biochemical indexes, i.e. serum or urinary monoclonal component (MC). These patients are often excluded by controlled trials, which enroll only cases with easily detectable indexes during the follow up. From 1999 to 2002 we have followed up 9 patients affected by nSM (4 f., 5m., mean age 60 yrs, r.54-70) all staged III A-B (multiple lytic or exhuberant lesions by conventional imaging procedures - CIP) and variable plasma cell marrow infiltration (mean: 16%, r. 1-65). At diagnosis, CIP revealed more focal lesions than Tc99m-sestaMIBI in 4 cases (17 vs. 12, respectively), while Tc99m-sestaMIBI was superior to CIP in 5 cases (33 vs. 24, respectively). Diffuse uptake of the tracer was present in 2/9 patients, with significant correlation with marrow plasma cell infiltration. All patients were treated with combined chemo- and radiotherapy; three were autotransplanted. Four patients attained clinical remission (absence of symptoms, normal marrow, no further lesions by CIP), lasting a mean of 23 mos. (r. 6-31). During remission, Tc99m-sestaMIBI showed only variable degree of diffuse uptake in absence of focality, which reappeared in relapse. Five patients never attained a complete disease control: in four of them CIP and Tc99m-sestaMIBI were concordant, but in one patient, who previously had showed focal tracer uptake, a bulky symptomatic tumor adjacent to L2 developed after chemotherapy was not detected by the tracer. In conclusion, Tc99m-sestaMIBI seems an excellent tracer in nSM, both at diagnosis and during the follow up. If confirmed, these data could prompt to reconsider the exclusion of this subset of patients from controlled multicentric studies.

PU166
UNUSUAL LEPTOMENINGEAL PROGRESSION OF MULTIPLE MYELOMA
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We describe the case of a 71 years old woman affected by multiple myeloma IgGk since May 2000. The initial stage of the disease was IA with monoclonal component of 2.8 g/dL and plasma cell infiltration of 70%, without osteolysis. After the first-line treatment with Melphalan (10 mg/m2 for 4 days/month) and Prednisone (80 mg/m2 for 4 days/month) for a total of 6 cycles the patient had a good response for which she continued maintenance therapy with Interferon α, stopped in June 2002 because of intolerance. Thus, she started an oral treatment with Melphalan (5 mg/week) stopped in December 2002 because she spontaneously broke her right tibia. In June 2003, she was hospitalised in the Neurology Department for an initial focalised epileptic crisis which became generalised. Two days before she experienced transversal abdominal pain. Two consecutive lumbar punctures revealed a bloody cerebrospinal fluid (CSF), but a diagnosis was
made only after the execution on an MRI which demonstrated a little mass, spontaneously hyperintense on T1 weighted images, localised on the surface of the cord at T10-T11 level. This formation was interpreted as a leptomeningeal localisation of Myeloma with an MR signal suggestive for recent bleeding. The CSF analysis showed the presence of the same Monoclonal Component of the blood and 8% of ANC identified as plasma cells (CD38+, morphology). So, we decided to begin a chemotherapy regimen based on the association of Vin-cristina (0.4 mg/day, continuous i.v. infusion), Adri-ablastina (9 mg/m^2/day, continuous i.v. infusion) and Desametasone (40 mg/day, i.v.) (VAD for 4 days), instead of antiepileptic therapy, which is still ongoing. At present, the patient is in a good state of health and the epileptic crisis no more appeared.

**PU167**

**ROLE OF BISPHOSPHONATES IN THALIDOMIDE-BASED THERAPY FOR REFRACTORY/RELAPSED MULTIPLE MYELOMA: EVALUATION OF TOXICITY AND EFFECTIVENESS**


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Thalidomide (Thal) displays a significant activity in relapsed/refractory Multiple Myeloma (MM) with cumulative response rates (RR) of 25-36% as a single agent. Its association with Dexamethasone (Thal/Dex) yields to RRs ranging from 50% to 60%. Several studies support the beneficial effects of bisphosphonates in MM, but there are few reports comparing the toxicity and efficacy of bisphosphonates in patients (pts) undergoing salvage therapy with Thal-based regimes. We report a retrospective analysis of 67 consecutive pts. from six southern Italy institutions undergone the Thal/Dex regime with or without a second (pamidronate)- or third (zoledronate)-generation bisphosphonate. All pts had advanced refractory/relapsed disease following different chemotherapy regimes (VAD, VMCP, MP), with/without radiotherapy and/or ASCT. Based on the treatment received, pts were divided in three groups: Thal/Dex (Thal 200 mg/day plus Dex 20 mg, days 1-4) (14 pts), Thal/Dex plus pamidronate (90 mg, q 28 days) (24 pts) and Thal/Dex plus zoledronate (4 mg, q 28 days) (29 pts). Study end points were to evaluate: a) the differential toxicity profiles (CTC-NCI) of bisphosphonates in pts receiving Thal/Dex; b) the potential role of bisphosphonates in improving anti-myeloma efficacy of the Thal/Dex combination. Response was evaluated according to SWOG and Nordic Myeloma Study Group. Analysis of prognostic factors among the groups was performed by log-rank test. To account for wider modifications of the monoclonal component (MC) in pts with higher and lower values at treatment start, a correlation test according to Gill et al. (Lancet 1985; 9: 567-569) was employed. The three groups were fully comparable for baseline parameters (Student T test). No statistically significant differences in frequency and severity of expected side effects (mostly G1/G2; G3/G4 <10%) were evidenced among the groups (i.e. G1/2 constipation 33%-41%, G1/2 neuropathy 17%-29%), except for G1/2 neutropenia which was only observed in pts receiving bisphosphonates (pamidronate, 12.5% vs zoledronate, 17.2%, NS). Only one case of deep vein thrombosis was recorded (zoledronate group). Details of responses (at a median follow-up of 8.1 months) according to treatment groups are shown in the table. Pts in all groups received a comparable percentage of Thal scheduled dose. Of the 36 responders only 2 (one each in the pamidronate and zoledronate groups) relapsed after 6 months of treatment. Most interestingly, however, time to best response was shorter for bisphosphonates-containing treatments (3.5±1.4 months, pamidronate; 3.4±1.4, zoledronate vs 6.16± 1.4 months, no bisphosphonate; p=0.059). The results obtained from this retrospective study suggest that adding bisphosphonates to Thal/Dex regime do not increase the frequency and severity of side-effects, but it seems to provide a faster attainment of maximal response. Data analysis at a longer follow-up is currently ongoing to ascertain whether bisphosphonates also result in a more sustained response. Based on this data, a prospective randomized trial comparing Thal/Dex to Thal/Dex/Zoledronate is currently ongoing at the same Institutions.
Starting from 1996, first line therapy for our patients aged less than 70 affected by advanced stage multiple myeloma (Salmon II or III) includes three DAV courses (adriamycin 50 mg/m² and vincristine 1 mg total dose in single bolus infusion on day 1 and dexamethasone days 1 to 4) followed by high dose melphalan (140 or 200 mg/m²) and one or two autologous stem cells transplantations (ASCT). Seventy-four such pts have been diagnosed in the period 1996-2002 (cases); fifty-three of them have received at least one ASCT; four did not receive this therapy due to comorbidity; nine were included in the program but they did not reach ASCT due to progression, toxicity of DAV courses or refractory disease; eight received MP as first line therapy because they were included in a randomized collaborative trial. A control group was created with 74 patients diagnosed in the years 1989-1995 (controls) matched for stage and age (±3 years); in this group 5 pts were initially treated with VAD-like courses, 32 with melphalan and prednisone, 35 with polichemotherapy (with two or more alkylating agents, without anthracyclines) 2 pts died before beginning chemotherapy. No differences were present between the two groups as to bone marrow plasma cell (mean 48.9% and 47.9%), classes (IgG: 49 and 46; IgA 16 and 15; IgD 0 and 2; BJ 7 and 9; NS 2 and 2), monoclonal component among IgG and IgA myeloma (mean 4.37 and 4.59 g/L). Results. Overall survival is significantly better for cases than for controls (median survival not yet reached vs. 39 months) (p=0.03). Statistically significant difference among cases and controls is maintained in Salmon stage II (p=0.03); among pts in stage III, the two curves are similar up to twenty months; after this time the curve of ‘cases’ becomes better. Conclusions. Our case-control study seems to agree with randomized studies, that show a survival benefit of ASCT when compared to conventional therapy in advanced stage multiple myeloma.
of liver enzyme (>2N). No patient had to discontinue NPT for metabolic complication. Median total days of hospitalisation were 27.2. Conclusion: While in most reported series patients received 30-35 Kcal/kg/day, our NPT was planned to administer to the patient between 1.1 and 1.2 times the basal energetic need. Patients received an average of 22.3 Kcal/kg/day (range 16-32 kcal/kg/day). Our TPN with reduced energetic support was proved effective to prevent proteic and energetic undernutrition and to avoid an increase of morbidity and mortality; an adequate NPT during hospitalization favourably affected infections. No patients suffered severe metabolic complications. Our results suggest that the use of nutritional support with reduced caloric intake is equally effective than hypercaloric regimens but it allows to reduce the costs and is less toxic.

**PU170**

TANDEM HIGH-DOSE THERAPY WITH PERIPHERAL BLOOD STEM CELLS TRANSPLANTATION (PBSCT) FOR PATIENTS WITH LYMPHOMAS

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Background. Single high-dose therapy (HDT) and peripheral blood progenitor cells (PBPC) is not sufficient to cure the majority of patients (pts) with poor prognosis lymphoma. Aims. To evaluate the feasibility and the efficacy of tandem HDT regimen supported by PBPCS in these patients. Design and Methods. After induction chemotherapy and collection of PBPCs (median CD34+ cells: 9.3×10⁶/kg) 21 pts with refractory/relapsed Hodgkin's disease (HD) and 21 pts with relapsed or poor prognosis non-Hodgkin's lymphoma (NHL) were given tandem PBSCT. The first conditioning regimen, consisted of Melphalan 200 mg/m² (PBSCT-1) and the second of BEAM (BCNU, Etoposide, ARA-C, Melphalan) (PBSCT-2) administered within 90 days from first reinfusion. Results. PBSCT-1 was supported by a median number of 4×10⁶ CD34+cells/kg. There was no toxic death. The median time to neutrophil recovery >500/µL was 6 days, and to platelet recovery >20000/µL was 3 days, respectively. Grade III-IV mucositis was observed in 85% of cases. The median number of days with fever > 38°C was 4 days. The mean number of platelet and erytocytes units transfused was two. Median hospitalization was 15 days. PBSCT-2 was performed after a median interval of 72 days (range 51-105), and supported by a median of 5×10⁹ CD34+cells/kg. Seven pts could not undergo second HDT: 4 for disease progression, and 3 for toxicity related to first procedure, respectively. Furthermore, we observed a toxic death during second procedure. No veno-occlusive disease (VOD) occurred. Median time to neutrophil and platelet recovery was 9 and 7 days, respectively. Grade III-IV mucositis and duration of hospitalization were identical to first procedure. The median number of days with fever > 38°C was 2.0. Complete remission (CR) rate was 57% after induction, 80% after PBSCT-1, and 90% after PBSCT-2. Conclusions. Tandem transplantation seems feasible in poor risk lymphomas. However, as 17% of pts could not undergo PBSCT-2, further investigation should aim to select suitable cases. As concern therapeutic outcome, the continuous progression towards a higher CR rate, encourages further exploration of this attractive strategy.

**PU171**

INTERMEDIATE DOSE ETOPOSIDE + LENOGRASTIM” IS AN EFFECTIVE PBPC MOBILIZATION SCHEDULE SUITABLE FOR OUTPATIENT ADMINISTRATION: A COMPARISON WITH CYCLOPHOSPHAMIDE 4 G/M² + G-CSF


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Cyclophosphamide 4 or 7 g/m² (CTX) + G-CSF is usually employed as PBPC mobilization. Such schedule, nevertheless, is associated to some morbidity: neutropenia and fever (52-97%); moreover it always require a period of hospitalization of various lenght (2-15 days). Searching for a mobilization schedules suitable for outpatient administration and with high mobilizing efficiency and low morbidity, we studied the scheme Intermediate dose Etoposide+ rh-gly-G-CSF (VP) administered at dose of 200 mg/m²/d.1-2-3 + lenogastim 16 mcg/kg/day from d.4 to the end of harvesting. Twenty patients affected with various hematological neoplasm (60% NHL, 28% HD, 12% others diagnosis) received as mobilization therapy Etoposide at intermediate dose + rh-gly-G-CSF. Results were compared to a group of 26 patients who received previously, in our Institution, CTX 4gr/m² + G-CSF 10 mg/kg/day. These two groups were not different in age, sex, time between last chemotherapy and PBPC mobilization and number of cycles of previous chemotherapy received. No differences were observed in blood volume processed during harvests and in mean number of aphaeresis performed (1,8 in VP16 group and 1,75 in CTX group). Percentage of patients that reached a peak of CD34+ cells > 20×10⁹/L and therefore underwent harvesting of PBPC was higher in patients mobilized with Intermediate dose VP+G-CSF” in respect to those who received CTX 4 g/m² + G-CSF”. 90.0% vs 61.5% (χ² p value = 0,04). Mean peak of peripheral blood CD34+
cells was more elevated in patients mobilized with “Intermediate dose VP-16”: 119.04/mm³ vs 45.8/mm³ (Mann-Whitney U-test: p=0.03). Similarly mean CD34+ cells in harvests was more elevated in patients mobilized using Intermediate dose VP-16: 10.2×10⁶/kg vs 6.8×10⁶/kg (p=0.01). Total Nucleated Cells were significantly higher in patients who received VP-16 while no differences were observed in harvested CFU-GM. Incidence of fever was lower in patients receiving Intermediate dose VP-16 + gly-G-CSF than in patients receiving "CTX + G-CSF, 5% and 19%, respectively, this difference did not reached, however, statistical significance (p value = 0.2). Length of hospitalization was significantly lower in Intermediate dose VP-16 mobilized group (0 vs 5.2 days; Mann-Whitney U-test p=0.0001). In conclusion our study shows that an outpatient PBPC mobilization schedule based on Etoposide at intermediate dose (600 mg/m²) + rh-gly-G-CSF 16 µg/kg has higher mobilizing efficacy and lower toxicity in comparison to CTX 4 g/m² + G-CSF10 µg/kg.

Table 1. Comparison of PBSC mobilization after VP-16 intermediate dose +G-CSF and after CTX 4g/m² + G-CSF.

<table>
<thead>
<tr>
<th></th>
<th>VP16 intermediate dose + G-CSF 16 mcg/m²</th>
<th>CTX 4 g/m² + G-CSF 10 mcg/m²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% successful mobilization</td>
<td>90%</td>
<td>61.5%</td>
<td>0.03</td>
</tr>
<tr>
<td>Day of hospitalisation</td>
<td>0.0</td>
<td>5.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Fever%</td>
<td>5.0</td>
<td>19</td>
<td>0.2</td>
</tr>
</tbody>
</table>

In conclusion in our study myeloid engraftment time of purged and unpurged transplants were comparable. After purged transplant exists a delay in platelets engraftment. Number of infused TNC is an important factor for myeloid engraftment either when

Table 2. Comparison of PBSC harvesting after VP-16 intermediate dose +G-CSF and after CTX 4g/m² + G-CSF.

<table>
<thead>
<tr>
<th></th>
<th>VP16 intermediate dose + G-CSF 16 mcg/m²</th>
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<td>5.0</td>
<td>19</td>
<td>0.2</td>
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</table>

In multivariate Cox analysis, the number of TNC infused in respect to median was a significant factor for LFS (p=0.03), independently of others factors such as FAB types. In conclusion in our study myeloid engraftment time of purged and unpurged transplants were comparable. After purged transplant exists a delay in platelets engraftment. Number of infused TNC is an important factor for myeloid engraftment either when
all patients are considered as a whole and in purged patient group, however not in unpurged patients. Number of infused TNC is an important factor also for LFS in all patients group and in unpurged group but not in purged patients.

PU173
HIGH-DOSE THERAPY IN THE ELDERLY WITH MULTIPLE MYELOMA: A SINGLE CENTRE EXPERIENCE
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Multiple myeloma (MM) is a disease which in almost 70% of cases involves patients older than 60 years and the number of patients is progressively growing. Being the life expectancy in western countries 78 years for men and 80 years for women we believe that the therapy of myeloma should be optimised in patients over 60 years in order to prolong survival, as it is for younger patients. In a prospective study we evaluated the feasibility of sequential therapy including high-dose Busulfan and Melfalan and PBSCT in patients with MM consecutively observed in our institution since 97. The entire program included VAD 4 monthly courses, Cyclophosphamide (CTX) 5gr/m² as mobilizing therapy of CD34 cells, collection of CD34 cells, minimum number of 2.5×10⁶/kg, Melfalan 120 mg/sqm single dose + Busulfan 12 mg/kg over 4 days, interferon alfa as maintenance therapy. The value of the disease was done at each step of therapy by bone marrow aspiration, value of serum proteins and Bence Jones protein in the urine; skeleton was done at the beginning and the end of the program. Follow-up was planned every two months with re-value of the same parameters. Side effects were monitored at each step of therapy by cardiac VEF, renal and liver function. Up to December 2002, 39 patients with MM and ages over 60 years (mean 66.7 years) entered the prospective value, 24 were males and 15 females. Most of patients had stage III (34 pts), the type of MM was IgG (21 pts), IgA (10 pts), Mycromolecular (5 pts) and hypo secreting (3 pts). The mobilization of CD34 was successful in 37 on 39 pts with a collection of mean number of CD34 cells 5.7×10⁶/kg (range 2.0 to 13.6). Response rate following each phase was: 23 pts had a reduction of M component or number of plasma cells following VAD, with a range between 30 to 80% reduction, other 7 patients showed a reduction of disease following high dose CTX and further response was achieved in those responding at the previous phase. Following PBSCT 31 pts (84%) had more than 50% reduction of disease and 12 pts (32%) had less than 10% of valuable disease. Lethal side effects consisted in an absence of hematological recovery in one pt with resistant MM who received 2.0×10⁶/kg CD34 cells (2.7%), an interstitial pneumonia by CMV infection (2.7%) and a septicaemia by Pseudomonas (2.7%). Non lethal side effects consisted of mucosytis grade III-IV in 40% of patients and fever in 35% of patients. Two patients died 4 and 5 months, respectively, following PBSCT because an acute renal failure following a septicaemia and an acute respiratory distress syndrome following a fever. At a mean follow-up of 30 months (range 6 to 74 months) of 34 patients valuable for response 16 patients remain in CR or PR (6 to 74 months), 14 patients progressed and died for MM, 2 patients are alive with progressive disease and 2 pts died for causes not related to mieloma. In conclusion the study demonstrate the feasibility of the procedure including high dose busulfan and melfalan in elderly with MM, further value has to be considered in order to minimize toxicity and to plan better conditioning regimens.

PU174
HIGH DOSE THERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN FOLLICULAR LYMPHOMA AT DIAGNOSIS
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Follicular lymphoma is characterized by an indolent course, with a 5-year survival of approximately 70%, and a median survival exceeding 10 years. Still, most patients ultimately die from the disease and innovative therapies should be considered. Autologous transplantation of ex vivo purged hematopoietic stem cells (HSC) has been performed in relapsed patients with promising results, and is currently being investigated as a front line treatment. Although controversial, this approach could be proposed to selected patients if treatment related toxicity was acceptable. Better supportive therapies as well as the widespread use of peripheral blood HSC and hematopoietic growth factors have rendered autologous transplantation a safer procedure. Furthermore, the availability of monoclonal antibodies that can be administered in vivo without additional toxicity could render the whole procedure less time consuming and might improve the results. At our institution, and in many others, treatment related mortality (TRM) following autologous HSC transplantation is less than 1% in patients with a good performance status. Thus, in 1995 we started a pilot study aimed to test autologous HSC transplantation in patients with FL at diagnosis. We have enrolled 24 patients with a median age of 49 (26-65). Most patients had stage IV disease (80%) and bone marrow involvement (74%). Patients achieving a complete response or a good par-
PU175

INCREASED DOSE OF BUSULPHAN IN THE BUMEL CONDITIONING REGIMEN FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION IMPROVES OVERALL RESPONSE RATE AND DISEASE-FREE SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction: High-dose chemotherapy conditioning regimens for autologous stem cell transplantation (ASCT) generally give similar results in multiple myeloma (MM). We compared two high-dose chemotherapy regimens: melphalan alone and melphalan plus busulphan. Methods: Thirty untreated patients with stage III MM were included in this study. The selection criteria included: age less than 70 years, creatinine less than 200 mg/dL, cardiac ejection fraction >50%, DLCO >50%, no active infection disease or other co-morbid conditions. Sixteen patients were treated with melphalan 180 mg/m² (arm A) and 14 with busulphan 16 mg/kg + melphalan 100 mg/m² (arm B) for ABMT conditioning. The median age at transplantation was 52.2 years (range 34-64) for arm A and 49.5 years (range 34-60) for arm B. In the arm A were included 9 male and 7 female and in the arm B 9 male and 5 female. All patients received 3 cycles of VAD and were mobilized with cyclophosphamide 7 g/m² and rhHuG-CSF (10 µg/kg b.w./die). The dose of CD34+ cells infused range from 1.5 to 15.6×10⁶/kg (median 9.2×10⁶/kg) for the arm A, and from 1.5 to 15.8×10⁶/kg (median 8.9×10⁶/kg) for the arm B. Results: After ASCT, days to achieve engraftment (neutrophils > 500/µL) were similar in the two arms (arm A: median 11, range 9-20; arm B: median 11, range 9-21); all the patients received rhHuG-CSF 10 µg/kg b.w./die after reinfusion of PBSC. No difference in the incidence of transplant-related infective and non-infective complications were evidenced in the two arms and no transplant-related deaths were observed. Hospitalization after transplant ranged from 12 to 18 days (mean 15 days) in both arms (p = n.s.). Better overall response rate was observed in the melphalan plus busulphan regimen (85% vs. 75%, p<0.05). MM progressed in two patients only in arm A. The 5-year overall survival with melphalan plus busulphan was 56% vs. 49% for melphalan alone. The median survival for the melphalan plus busulphan group was 126 months vs. 108 months for melphalan alone (p=0.6). The median disease-free survival (DFS) was better for melphalan plus busulphan (120 months) than for melphalan alone (72 months) (p<0.05). Conclusion: These two conditioning regimens, we used for ASCT in multiple myeloma, have a similar anti-myeloma effect. However, the conditioning regimen with Busulphan (16 mg/kg) and Melphalan (100 mg/m²) was as well tolerated as the conditioning with melphalan alone, and gave better results in terms of response to treatment and disease-free survival, but had no effect on the overall survival that was not significantly increased in arm B.
We compared the outcome and the engraftment kinetics of two consecutive matched group of patients receiving different schedules of GF after ASCT. Among 65 patients with multiple myeloma or lymphoma, 32 received G-CSF plus EPO starting the day after ASCT (group A), and 33 received G-CSF (group B) starting the day +5 after PBSC reinfusion. The two groups were matched for the main characteristics including age, diagnosis, median number of CD34+ reinfused, conditioning regimens, PS and number of previous chemotherapy regimens. Patients of group A achieved a PMN count >5x10^9/L at the day 10 (10-10), PLT>20x10^9/L at day 13 (13-14), PLT>50x10^9/L at day 17 (15-19) compared with day 11 (10-12) for PMN>0.5x10^9/L, day 14 (12-16) for PLT>20x10^9/L and day 24 (7-41) for PLT>50x10^9/L respectively. The median number of days with PMN<100x10^9/ml and PMN<500x10^9/ml was 3 (0-6) and 5 (1-9) in the group A, compared with 5 (1-22) and 7 (3-23) in the group B (p<0.0001 and p=0.001 respectively). Also the transplant outcome was significantly better in patients receiving EPO+G-CSF (group A) with a median of 1 PLT units (0-5) transfused, 0 (0-6) RBC transfusion units, compared with 2 PLT units (0-9) and 2 RBC units (0-8) in the group B (p=0.001 and p<0.0001 respectively). Also the median number of days with fever >38°C was 0 (0-8) compared with 1 (0-9) (p=0.01) and days of i.v. ATB therapy were 0 (0-8) and 1 (0-9), respectively in group A and B (p=0.01). Finally the median number of days in hospital from the day of reinfusion was 2 (2-19) in group A and 14 (6-28) in group B (p<0.0001). In conclusion this approach allows to further reduction of the aplastic phase after ASCT and the costs of this procedure.

**PU177**

**EFFECTIVENESS AND TOXICITY OF TWO DIFFERENT CHEMOTHERAPY PROGRAMS IN AUTOLOGOUS PERIPHERAL STEM CELLS TRANSPLANTATION IN PATIENTS WITH NON-HODGKIN LYMPHOMAS**

Colaemma A, Borin LM, Piotelli P, Rossini F, Maffe' P, Terruzzi E, Pogliani EM

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In this retrospective study, we wanted to compare the therapeutic efficacy and the toxicity of two different chemotherapy programs including both autologous peripheral stem cells transplantation (PBSC) in a cohort of patients diagnosed with Non-Hodgkin lymphomas (HG-NHL). Thirtyseven patients were divided in two groups (group A and group B); 21 patients (Group A), fourteen at onset and seven relapsed received high dose sequential chemotherapy including: adriamicine 50 mg/m^2 (g 1), vincristine 1.4 mg/m^2 (g 1-8-15), prednisone 40 mg/m^2 for 21 days; cyclophosphamide 7 g/m^2 and G-CSF 5 µg/m^2 followed by CD34+ cells mobilization/HD-methotrexate 8 g/m^2 and vincristine 1.4 mg/m^2 // HD-etoposide 2 g/m^2 and metilprednisolone 180 mg; conditioning regimen consisted of: mitoxantrone 60 mg/m^2 (d1) and melphalan 180 mg/m^2 (d4). A median number of 8.5x10^6/kg autologous CD34+ cells were reinfused as rescue. Sixteen patients (Group B), only first relapse, received standard dose chemotherapy comprehensive of 4-6 CHOP/CEOP cycles (in CEOP cycles epirubicine was given instead of adriamicine); two cycles of MAD (mitoxantrone 8 mg/m^2 (d 1-2-3), ARA-C 2 g/m^2 x 2 (g 1-2-3) and desametasone 8 mg/m^2 (d 1-2-3) and G-CSF 5 µg/m^2); CD34+ cells were collected after the second MAD cycle; BEAM protocol was used as conditioning regimen. A median number of 8.7x10^6/kg autologous CD34+ cells were reinfused as rescue. The median time to hematopoietic recovery was similar in both groups: 13 days, group A and 11 days, group B. There wasn't statistically significant difference in overall survival (OS) and disease free survival (DFS) between the two groups (5 years follow-up): OS of Group A was 47.6% versus 50% of Group B, DFS of Group A was 43% versus 44% of Group B. By contrast acute and long term toxicity was worst for the cohort of patients that received high dose sequential chemotherapy (Group A): we observed mucositis, grade WHO II/IVo, in 45% of Group A patients versus 27% of Group B patients. Moreover, in group A, 1 patient developed an RAEB (36 months post-transplant), 1 patient Hodgkin’s lymphoma (24 months post-transplant), 3 patients an assonal neuropathy (2 months post-transplant), 2 patients MGUS (8 months post-transplant) and finally 1 patient developed hypotyroidism (36 months post-transplant); in group B, 1 patient developed an AML (30 months post-transplant) and 1 patient had a reactivation of an hepatitis infection (3 months post-transplant).
Autologous PBSC transplant (APBSCT) in multiple myeloma (MM) has become a standard treatment for most patients; there are many evidence-based recommendations based on the evidence presented that APBSCT is preferred to standard chemotherapy as de novo or salvage therapy; but it has a minimal role on duration of remission so that a post transplant therapy for patients in CR or PR is needed. Thalidomide has recently emerged as an active drug in about one-third of MM patients, who had usually been heavily pre-treated or had relapsed after high-dose therapy. At the same time, interferon-α (IFN-α) as maintenance treatment is associated with improved survival after APBSCT in patients with multiple myeloma. Methods: with the intent to evaluate the impact of post transplant maintenance therapy on disease free survival (DFS), we have randomized trials using thalidomide in combination with interferon are needed.

The median age at the time of ASCT was 62 years (range 60-67). In 8 patients (5 CR and 3 PR) ASCT was performed as part of the front line therapy while in 6 (3 sensitive relapse, 1 primary refractory, 2 progressive disease) as part of the salvage therapy. The source of stem cells was bone marrow in 2 cases (14%) and peripheral blood in 12 (86%). With the exception of 1 patient with HL who received BEAM, all the other patients were treated with the BAVC conditioning schedule (carmustine, cytotoxic-arabinoside, etoposide, PU179 HIGH-DOSE THERAPY WITH STEM CELL RESCUE IN ELDERLY PATIENTS WITH AGGRESSIVE POOR RISK LYMPHOMAS: A REVIEW OF 14 CASES Fili C, Zaja F, Sperotto A, Patriarca F, Cerno M, Calistri E, Skert C, Damiani D, Kikic F, Mestroni R, Fanin R Clinica Ematologica, Policlinico Universitario, Udine, Italy

The treatment of elderly patients with lymphoma is an ever more difficult challenge. The increasing mean life expectancy of the population, together with a rising incidence, in these last decades, of high-grade non-Hodgkin lymphomas (NHL) particularly in the adult population, has stimulated the application of more intensive therapeutic strategies also in elderly patients. We report here the results of a retrospective analysis performed in 14 patients older than 60 years who underwent, between May 1998 and June 2003, high dose therapy (HDT) and autologous stem cell transplantaion (ASCT) as part of consolidation or salvage treatment. The patients’ clinical characteristics are summarized in Table 1.

<table>
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<tr>
<th>Pt.</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Stage</th>
<th>IPI</th>
<th>Bulky</th>
<th>Therapies Months</th>
<th>Status</th>
<th>Status</th>
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<td>1</td>
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<td>4B</td>
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<td>19</td>
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<td>11 PR CR No</td>
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<td>m</td>
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<td>4B</td>
<td>LI</td>
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<td>15 CR CR No</td>
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<td>11 CR CR No</td>
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</table>

NCL: mantle cell lymphoma; HL: Hodgkin’s disease; B-DLCL: diffuse large cell; peripheral lymphoma; FOL 2: follicular lymphoma grade 2; FOL 3: follicular lymphoma grade 3; ALC: anaplastic large cell lymphoma.
cyclophosphamide). The median time between diagnosis and ASCT was 15 months (range 8-65) and the median number of CD34+ stem cell reinfused was 2.7 × 10^6/kg (range 0.57-6.5). None of patients had clinical or instrumental (as documented by ECG and echocardiography) signs of heart disfunction before HDT. All patients after ASCT received antibacterial and antifungal prophylaxis with levofloxacin and itraconazole and G-CSF up to hematological recovery. Median time for neutrophils major then 0.5 × 10^9/L and platelets major then 20 × 10^9/L was 10 days (range 9-20) and 11 days (range 8-22), respectively. Five patients (36%) experienced grade III-IV (WHO) mucositis. Infectious complications during the phase of cytopenia included: fever of unknown origin 7 patients (50%), pneumonia 1 patient, abdominal abscess 1 patient, Gram + bacteremia 5 patients. Two patients developed an acute heart failure on day +2 and +3 after ASCT. Long-term complications after ASCT included 1 herpes zoster, 1 ventricular dysfunction and 1 fatal fungal infection at month +22. After a median follow-up of 12 months after ASCT (range 1-30), 12 patients are alive (86%); in 2 patients the follow-up is still too short to evaluate the evaluation of response after ASCT. Two patients died, 1 of infectious complication and 1 for progressive lymphoma. Our preliminary results indicate that HDT with stem cell rescue is a rather toxic therapeutic procedure in elderly patients. Better selection of patients and the use of different conditioning regimens (in particular containing less potentially cardiotoxic drugs) seem advisable when an intensification of the therapeutic program with HDT is considered.

PU180
TRANSPORT OF AUTOLOGOUS HEMATOPOIETIC STEM CELLS IN PATIENTS WITH HODGKIN'S DISEASE: A SINGLE-CENTER EXPERIENCE
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We conducted a retrospective analysis of 71 Hodgkin's disease (HD) patients (pts) submitted to autologous hematopoietic stem cell (HSC) transplantation at Bone Marrow Unit of our Center, between 3/1986 and 10/2002. In this study we analyzed the characteristics and the outcome of patients, overall survival (OS), event free survival (EFS) and transplant complications. Eligible patients were 16 to 58 years old; they were refractory, relapsed or partially responding to first line or salvage treatment or with advanced disease at diagnosis (bulky or extranodal disease). At diagnosis 80% of patients were in Ann Arbor clinical stage III-IV; 32% had bulky disease; 60% B symptoms and 28% had extra-nodal disease. Most of them (80%) received 3 or more regimens before transplant and 62% received radiotherapy. Median time from last chemotherapy to high dose regimen was 11 weeks (range 3-34). Fifty-six pts were conditioned with CVB and 15 pts with BEAM; the graft consisted of bone marrow (21 pts) or peripheral blood stem cell (50 pts). At the time of HSC transplant 28/71 pts were in CR, 16/71 in PR, and 27 were considered non responder. A total of 62 (87%) pts responded to HSC auto-transplant with 53 CR and 11 PR. After a median follow-up of 113 (range 1-199) months, OS was 54% at 5 years and 38% at 10 yrs with an estimate EFS of 60% at 5 years. The regimen related mortality was 4.2%. According to chemosensitivity prior to transplant, patients were stratified in a three group: chemosensitive (43%) multiple relapse (39%), and resistant (16,9%); the 5 yrs OS was 76% for sensitive group vs 58% for multiple relapse; at 5 yrs all resistant pts were died (p= 0.001); the EFS was 78% for sensitive group vs 63% for multiple relapse. Both univariate and multivariate analysis indicated pre HSC chemosensitivity as the most important prognostic factor influencing OS and EFS. All refractory patients died of progression of disease within 4 years. Extranodal disease at diagnosis was not a negative prognostic factor if associated to chemosensitive disease: 10 yrs OS in subset of sensitive pts with extranodal disease was 67%, vs 21% for those who had refractory or multiple relapse disease. Of the 53 pts in CR at day +100, 43 (81%) are still alive; 10 patients died within 5 years: 7 of relapse and 3 of regimen related toxicity (one pulmonary fibrosis and cardiomyopathy, one B virus hepatitis, one pulmonary infection). Two patients (2,8%) experienced, two and four years after transplant, a myelodysplastic syndrome; one patients was affected by uterus carcinoma in situ 1 yr after transplant and one by adenomatous polyps of colon 3 years after transplant, with cumulative incidence of secondary malignancy of 5.6%. Our data confirm that HD pts who fail to respond to first line chemotherapy or who have advanced disease may benefit from high dose chemotherapy followed by autologous stem cell transplantation. Novel strategies are needed to reduce the risk of relapse.
Treatment with fludarabine (FAMP) for patients with B-cell chronic lymphocytic leukemia (B-CLL) induces an overall response of about 60-70%, with a complete remission rate of 20-30%. However, the median time at relapse is of 20 to 30 months. Thus, there is a need for new therapeutic approaches to reach a longer duration of response. High-dose chemotherapy with autologous stem cell transplantation has been suggested, but residual leukemic cells contaminating graft may contribute to relapse. Aim of this study was to use the Matthera, a chimeric humanized anti-CD20 monoclonal antibody, to purge the graft of B-CLL patients who achieved clinical complete remission (CR) or nodular partial remission (nPR) after treatment with FAMP. The results of this new purging strategy are reported for 3 patients with advanced B-CLL. The patients' characteristics are summarized in Table 1.

<table>
<thead>
<tr>
<th>Pts.</th>
<th>Age/sex</th>
<th>Stage before treatment (Binet)</th>
<th>Previous Treatment</th>
<th>Status Before Tx</th>
<th>CD19+ CD5+ contamination of ASCT (×106/kg)</th>
<th>Follow-up by ASCT (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.M</td>
<td>49/M</td>
<td>C</td>
<td>FAMP x 4</td>
<td>nPR</td>
<td>15.01</td>
<td>53</td>
</tr>
<tr>
<td>S.U.</td>
<td>54/M</td>
<td>B</td>
<td>FAMP x 4</td>
<td>nPR</td>
<td>24.32</td>
<td>31</td>
</tr>
<tr>
<td>D.P.V</td>
<td>45/F</td>
<td>B</td>
<td>FAMP x 4</td>
<td>CR</td>
<td>0.77</td>
<td>14</td>
</tr>
</tbody>
</table>

All patients were treated with FAMP (25 mg/m2 iv for 5 days) repeated every 4 weeks for 4 cycles. At the time on transplant, 2 patients were in clinical nPR and 1 patient in clinical CR, but none in immunophenotypic remission. Peripheral blood stem cells (PBSC) mobilization was made using Cyclophosphamide (3.5 g/m2/day) plus VP-16 (300 mg/m2/day) for 2 days followed by daily rhG-CSF (5 mg/kg s.c.). The flow cytometric analysis of PBSC buffy coats showed, for all patients, a contamination by ASCT (×106/kg) = 2, platelet supports = 1). Conditioning regimen was BEAM (BCNU, etoposide, ara-c, melphalan) protocol. Purging procedure: PBSC were rapidly thawed at 37°C and normal saline (volume 1:1) was added drop by drop. After centrifugation (2500 rpm for 15 min., at 4°C) the supernatant was discarded and the pellet was resuspended with 100 mL of RPMI 1640 medium. Matthera was added (100 mg for each bag) and after 30 min. of incubation (at room temperature) the cells were washed with RPMI 1640 medium, to discard the excess of Matthera and finally infused. Thus, all patients received PBSC in which Matthera has been bonded on the surface of B lymphocytes. Results: CD19+ peripheral blood lymphocytes could not be detected at 3, 4 and 4 months. All patients are in clinical and flow cytometric CR at 53, 31 and 14 months.
with decreased therapeutic tolerance in the host or adverse biology of the underlying disease. Recent data have shown that autologous stem cell transplantation (ASCT) is only feasible in a minority of elderly AML patients because of lower rate of complete remission (CR), more frequent occurrence of early relapse and inelegibility due to toxic effects of induction/consolidation chemotherapy. In particular, infection from HCV represents a major impairment to stem cell transplantation, mainly in elderly individuals. Here we report the case of a 61 years old HBSAg+ AML patient, who received ASCT in CR2 after antiviral therapy with Lamivudine, and IFN because of a concomitant HbsAg+ and HCV+ hepatitis. Of note, CR2 was achieved with gentuzumab ozogamicin (GO). The patient was diagnosed as having AML (FAB M4) on march 2002 Karyotype was normal as were liver and renal parameters. Positivity for HBSAg was found. Induction treatment included with continuous infusion of Fludarabine plus cytarabine was administered and, after CR achievement, a consolidation with the same regimen was given with collection and cryopreservation of 12×10⁹/kg CD34+ cells. Antiviral prophylaxis with lamivudine (100 mg/daily) was started before induction. On june 2002, jaundice appeared with concomitant elevation of SGOT and SGPT as well as positivity for HCV (HCV RNA 130570c/mL). The patient was started on PEG-Interferon (80 IU/week) and biochemical and virologic remission was obtained after two months of therapy. Antiviral treatment was stopped after 4 months. On January 2003, hematologic relapse was observed. The patient was treated with Gentuzumab Ozagamycin at 9 mg/m² on days 1 and 21 with achievement of CR2. HCV antibodies and RNA were negative and two months later the patients was autografted with a conditioning regimen consisting of continuous infusion idarubicin (20 mg/m² on days -12,-11) and busulphan 4 mg/kg on days -4 to -2. Hematopoietic recovery was fast and the patient is in sustained CR2 after antiviral therapy with Lamivudine and IFN because of a concomitant HbsAg+ and HCV+ hepatitis. Relapse occurred in October 1993 but a second CR was obtained and consolidated with an autologous bone marrow transplantation performed on April 18th 1994. The conditioning regimen was Busulphan 14 mg/kg plus Cyclophosphamide 120 mg/m². Granulocyte engraftment occurred on day +36, platelets engraftment on day +42. The patient did not experience significant toxicity. Relapse occurred 5 months later and the patient died in November 1994 for progressive disease. 2. UPN 202: female, 55 years old, affected by AML M4 since March 10th 1993. She achieved CR and in the same year was nefrectomized for renal cancer. She underwent an autologous bone marrow transplantation on August 18th 1994, conditioned with Busulphan 14 mg/kg plus Cyclophosphamide 120 mg/m². Granulocyte recovery occurred on day +18, platelets recovery on day +34. Any significant toxicity was observed. Relapse occurred 7 months later and the patient died in April 1996 for progressive disease. 3. UPN 650: male, 60 years old, previously nefrectomized for a parent-to-child kidney donation; he was referred to our Unit for a high grade NHL in June 2001, treated with splenectomy and CHOP. In the meantime, the patient underwent a lobectomy for lung cancer. He achieved CR consolidated with peripheral blood autologous stem cell transplantation conditioned with ICE (Ifosfamide 3000 mg/m² on days -6,-5,-4,-3; VP16 300 mg/m² bid on days -6,-5,-4; Carboplatin 500 mg/m² on days -6,-5,-4). Granulocyte engraftment occurred on day +10, platelets engraftment on day +11. The patient did not experience any significant toxicity and he is still alive and in CR. These three cases demonstrate the feasibility of high-dose chemotherapy with standard conditioning regimen followed by autologous stem cell rescue in monorenal patients, without significant toxicity. We conclude that the monorenal condition has not to be considered an exclusion criterion for autologous transplantation.

**PU184**

FEASIBILITY OF AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN NEFRECTOMIZED PATIENTS: REPORT OF THREE CASES WITH STANDARD CONDITIONING REGIMEN


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We describe three cases of monorenal patients treated with autologous stem cell transplantation at Florence BMT Unit between 1994 and 2001. 1. UPN 185: female, 38 years old, affected by AML M1 since October 10th 1991. After achieving CR, in 1992 she was nefrectomized for xantomatous flogosis. Relapse occurred in October 1993 but a second CR was obtained and consolidated with an autologous bone marrow transplantation performed on April 18th 1994. The conditioning regimen was Busulphan 14 mg/kg plus Cyclophosphamide 120 mg/m². Granulocyte engraftment occurred on day +36, platelets engraftment on day +42. The patient did not experience significant toxicity. Relapse occurred 5 months later and the patient died in November 1994 for progressive disease. 2. UPN 202: female, 55 years old, affected by AML M4 since March 10th 1993. She achieved CR and in the same year was nefrectomized for renal cancer. She underwent an autologous bone marrow transplantation on August 18th 1994, conditioned with Busulphan 14 mg/kg plus Cyclophosphamide 120 mg/m². Granulocyte recovery occurred on day +18, platelets recovery on day +34. Any significant toxicity was observed. Relapse occurred 7 months later and the patient died in April 1996 for progressive disease. 3. UPN 650: male, 60 years old, previously nefrectomized for a parent-to-child kidney donation; he was referred to our Unit for a high grade NHL in June 2001, treated with splenectomy and CHOP. In the meantime, the patient underwent a lobectomy for lung cancer. He achieved CR consolidated with peripheral blood autologous stem cell transplantation conditioned with ICE (Ifosfamide 3000 mg/m² on days -6,-5,-4,-3; VP16 300 mg/m² bid on days -6,-5,-4; Carboplatin 500 mg/m² on days -6,-5,-4). Granulocyte engraftment occurred on day +10, platelets engraftment on day +11. The patient did not experience any significant toxicity and he is still alive and in CR. These three cases demonstrate the feasibility of high-dose chemotherapy with standard conditioning regimen followed by autologous stem cell rescue in monorenal patients, without significant toxicity. We conclude that the monorenal condition has not to be considered an exclusion criterion for autologous transplantation.

**PU185**

FRACTIONATED TOTAL BODY IRRADIATION AND MELPHALAN FOLLOWED BY ASCT FOR PATIENTS WITH ACUTE MYELOID LEUKEMIA IN FIRST OR SUBSEQUENT COMPLETE REMISSION

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It is well known that autologous stem cell transplantation (ASCT) is an effective treatment strategy for
Acute myeloid leukemia (AML) in first or subsequent complete remission (CR). Unfortunately the golden standard conditioning regimen is still not well defined. In this study we report our experience of 34 AML patient with fractionated TBI (12 Gy in 6 fraction over 3 days) and Melphalan (140 mg/m² in a single dose) as preparative regimen for ASCT. Nineteen patients were male and 15 female. The median age was 37 years (range 3-55 yrs). Twenty-nine pts were in first CR and 4 in second CR. Twenty patients received PBSC. GCS-F was used from day +1 up to engraftment. Cytogenetics was performed in all patients and 11 of these showed a favourable karyotype including t(8;21) and inv16. The median interval between diagnosis and ASCT was 4 months. All pts achieved hematopoietic recovery in a median time of 11 days (range, 9-35) for neutrophils and 17 days (range,10-120) for platelets. Treatment related toxicity consisted mainly of oral and gastrointestinal mucositis. No transplant related deaths were observed. Fifteen (44%) of 33 patients relapsed after a median time of 6 months from ASCT (range,3-18); twelve/15 patients died: 10 pts of leukemia and 2 pts of transplant related complications after a subsequent mismatched transplant. At present, the remaining 3 patients are alive in second CR: one after a second ASCT (+71 months), one after an allogeneic BMT (+19 months) and one after FLAG therapy (+2 months). Nineteen patients (56%) are in continuous CR (16 in first and 3 in second) with a median follow-up of 50 months (range,2-127). The 2 year Kaplan-Meyer overall survival is of 62%. In conclusion our data suggest that TBI + Melphalan is a safe and well tolerable conditioning schedule with an effective anti-AML activity.

Pu186
CLINICAL VALUE OF POSITRON EMISSION TOMOGRAPHY FOR MONITORING PATIENTS AFFECTED BY MALIGNANT LYMPHOMA AFTER HIGH DOSE THERAPY AND STEM CELLS TRANSPLANT
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Fluorine-18 fluorodeoxyglucose (18FDG) is a glucose analogue used in oncology imaging as a marker of glycolysis, which is enhanced in tumours compared to normal tissue. Several studies have shown the effectiveness of 18FDG positron emission tomography (18FDG-PET) in the post-treatment evaluation of lymphomas and reported that it has a high predictive value for the differentiation between active tumour and fibrosis in patients with a residual radiological mass. PET evaluation before stem cell transplantation is also considered a very sensitive marker of stem cell transplantation outcome. We evaluate the predictive value of 18FDG-PET in 35 patients affected by non Hodgkin’s (26 pts) and Hodgkin disease (9 pts) after high dose chemotherapy and autologous stem cell transplantation. There were 21 males and 14 females, with a median age of 41 years (range 22-63). Twenty four patients received unmanipulated PBSCT, 6 patients received CD 34+ selected PBSC. CD34+ selection was carried out using CliniMacs device (Miltenyi Biotech GmbH, Bergish-Gladbach, Germany). We performed a diagnostic evaluation including clinical examination, laboratory screening, CT (total body) and bone marrow biopsy before and 3 months after transplantation and PET restaging was performed at 100 days after transplant. Twelve patients had a positive PET scan 100 days after transplantation. Among them, 5 patients showed active disease (42%): 2 patients rapidly progressed after transplantation and 3 patients relapsed at 5,10 and 20 months respectively. Seven patients did not show relapse of their disease during follow-up at a median time of 19 months (range 10-36). One patient with a positive PET scan had no evidence of active lymphoma but a diagnosis of second neoplasia was made at months. Twenty-three patients had a negative PET scan 100 days after transplantation. Among them, 4 patients (17%) relapsed at 5, 6,11 and 12 months respectively. A positive PET scan was detected during follow-up in one patient at 11 months after transplantation. The correlation between PET evaluation and clinical outcome was not statistically significant (p=0.11). Overall median follow-up was 17 months (range 6-51). The 2 years overall survival (OS) rates were 77% in PET+ patients and 90% in PET- patients (p=0.55). The 2 years FFP (freedom from progression) rates were 55% in PET+ patients and 79% in PET- patients (p=0.24). Considering the low sensitivity of low grade lymphomas to PET we then excluded patients transplanted for low grade lymphomas. The correlation between PET evaluation and clinical outcome was again not statistically significant (p=0.25). OS at 2 years was 87% in PET+ patients and 88% in PET- patients with a p=0.88. FFP at 2 years was 53% in PET+ and 75% in PET- (p=0.32). We did not find any statistically significant difference in the overall survival and in freedom from progression according to the results of PET scan at 100 days after autologous stem cell transplantation. The lack of significance might be probably due to the small number of patients and to the length of follow up. Further studies are required to allow the exact role of of PET after autologous stem cell transplantation.
PU187 TANDEM HIGH DOSE CHEMOTHERAPY (HDC) WITH PERIPHERAL BLOOD PROGENITOR CELL (PBPC) SUPPORT IN PATIENTS WITH METASTATIC BREAST CANCER RESPONSIVE TO INDUCTION CHEMOTHERAPY

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To determine feasibility and efficacy of tandem HDC with PBPC rescue in women with metastatic breast cancer 21 patients (pts) not previously treated for metastatic disease were enrolled. All pts received an induction phase consisting of 4 courses of epirubicin 90 mg/m² + 5-fluorouracil 600 mg/m² + cyclophosphamide 600 mg/m² (6 pts) or docetaxel 75 mg/m² + epirubicin 120 mg/m² (15 pts). Patients with response to induction were eligible to proceed with HDC. The sixteen pts that obtained a response (7 CR and 9 PR), received one additional course of the same induction treatment plus G-CSF 5 mcg/kg/d to collect PBPC. Two HDC using different non-cross-resistant agents consisted of high dose melphalan (200 mg/m²) as first course and ICE regimen (Ifosfamide 12 g/sm, carboplatin 18 AUC and etoposide 30 mg/kg) as second one. The median interval between HDC 1 and 2 was 10 days. Cycle 1 was omitted in two patients that did not collect a minimum of CD34+ cells to support both HDC. A median of 4.7 (range 0.5-7.3) and 5.7 (range 3-9.1) x10⁶/kg CD34+ cells were reinfused after each course of HDC respectively. Positive selection of CD34+ cells was performed in 12 patients. Hematologic reconstitution was rapid after each HDC with absolute neutrophils counts >500/µL and platelet counts >20,000/µL after a median of 10.5 and 11.5 days after the first and 9.5 and 12 days after the second course respectively. No treatment related death was observed. Extra-hematologic toxicities no exceeded WHO grade II: emesis, mucositis, diarrhea and fever were the most common ones. No significant differences were found between first and second transplant. After HDC 4 out 9 PR were converted in CR. The median progression-free survival (PFS) and the median overall survival (OS) from diagnosis were 35 months and 48 months respectively. Our results suggest that two cycles of HDC consisting of high-dose melphalan and ICE with PBPC support are feasible, safe and tolerable. The observed PFS and OS are promising and are better than expected following single HDC.

PU188 CONCURRENT RITUXIMAB AND INTENSIFIED CHEMOTHERAPY MEGACEOP + MAD FOLLOWED BY HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRAPSULATION (ASCT) IN HIGH RISK B-DIFFUSE LARGE CELL LYMPHOMA (B-DLCL): A MULTICENTER PILOT STUDY

Vitolo U,1 Liberati AM,2 Cabras MG,2 Pavone E,4 Salvi F,4 Rota-Scalabrini D,4 Angelucci E,1 Boccomini C,1 Chiappella A,1 Di Vito F,1 Freilone R,1 Genua A,2 Orsucci L,1 Pogliani E,3 Pregno P,3 Rossi G,3 Tonso A,3 Gallo E

Introduction: the addition of Rituximab to standard chemotherapy CHOP improves the outcome of advanced stage B-DLCL, however patients at high risk continue to do worse. The combination of Rituximab with intensified chemotherapy is still poorly studied so far. Patients and methods: from January 2001 to May 2003, 45 previously untreated patients <60 years affected by B-DLCL at age-adjusted IPI intermediate-high (IH) or high (H) risk and/or with bone marrow (BM) involvement were enrolled. They were treated with an intensified chemo-immunotherapy regimen R-MegaCEOP (Rituximab 375 mg/m² day 1, Ctx 1200 mg/m² + Epi 110 mg/m² + VCR 1.4 mg/m² day 3 and PDN 40 mg/m² day 3 to 7) every 14 days with G-CSF support for 4 courses±2 DHAP; patients in CR or PR received two courses of intensified chemotherapy R-MAD (Mitoxantrone 8 mg/m² + Ara-C 2000 mg/m²/12h + Dexamethasone 4 mg/m²/12h days 1 to 3 and Rituximab 375 mg/m²/ day 4 and before peripheral blood stem cell harvest as in vivo purging) followed by ASCT with BEAM as conditioning regimen±T. All pts were given antibacterial and anti-fungal prophylaxis throughout the whole treatment. Results: the trial is ongoing. Up to now 23 pts are evaluable: median age 43 years (27-58); 13% were at LI, 43% at IH and 44% at H risk according to IPI; 30% had BM involvement and 70% LDH level >normal. Complete remission was achieved in 74% of the pts, PR in 14%, 18% did not respond and 4% (one patient) died of toxicity. Seven pts were not autografted: 2 because of inadequate PBSC yield, 4 progressed during therapy and one died of toxicity. Infections (WHO ≥2) were reported in 8 patients: 2 pts with bacterial pneumonia, 2 pts with interstitial pneumonitis, one with lung aspergillosis, one with perianal abscess and two with late CMV reactivation after ASCT. One pts died of toxicity due to interstitial pneumonitis during neutropenia after R-MAD. One patient developed a bacterial meningitis 20 months after
ASCT that resolved quickly. Conclusions: the preliminary results of this trial suggest that the concurrent use of Rituximab with intensified chemotherapy followed by high dose chemotherapy with ASCT support is feasible and effective to achieve a high CR rate in B-DLCL at poor prognosis. However the incidence of infections is worrisome and these patients need to be carefully monitored in order to avoid severe toxicities and to further improve their outcome.

PU189
AN "AGE ADJUSTED" HIGH DOSE CHEMOTHERAPY (HDC) WITH ASCT IS WELL TOLERATED AND EFFECTIVE IN ELDERLY PATIENTS WITH AGGRESSIVE NON HODGKIN'S LYMPHOMA (NHL).

Pregno P,1 Botto B,1 Liberati AM,2 Pavone E,3 Salvi F,1 Badone M,2 Boccomini C,1 Freilone R,1 Degli Angeli P,2 Larizza E,1 Levis A,4 Orsucci L,1 Gallo E,1 Vitolo U,1 on the behalf of the Intergruppo Italiano Linfomi
1UOA Ematologia 2, ASO S. Giovanni Battista Torino;
2Medicina Interna Università di Perugia; 3Ematologia, Università di Bari; 4Ematologia ASO SS Annunziata e Biagio di Alessandria; 5Medicina Interna Ospedale Degli Infermi di Biella, Italy

Introduction: the majority of elderly patients (>60 yrs), who are usually considered not eligible for HDC, might tolerate ASCT with an "age-adjusted" regimen. Patients and Methods: Pts aged between 61-70 yrs with aggressive NHL at diagnosis or in relapse/progression after standard chemotherapy, were treated with: A) 6 weeks of P-VEBEC chemotherapy (at diagnosis) and/or 2 courses of DHAP (at 75% of the regular dose) for pts with slow response or in relapse/progression; B) intensification with Mitoxantrone 8 mg/m² + high-dose ARA-C 1500 mg/m²/12hr + Dexamethasone 4 mg/m²/12hr for 2 days and G-CSF 5 mcg/Kg/d from day +3 to harvest peripheral blood stem cells (PBSC); C) ASCT conditioned by BCNU 200 mg/m² day -6, ARA-C 200 mg/m²/12hr + VP-16 100 mg/m²/12hr days -5 to -3, Melphalan 120 mg/m² day -2, reinfusion of at least 5x10⁹/kg CD34+, G-CSF 5 µg/Kg from day +1 to engraftment. Results. Since January 1998, 50 pts have been enrolled: 27 males, 23 females, 29 B-DLCL de novo, 13 transformed follicular, 3 mantle cell, 3 ALCL and 2 peripheral T-cell. Thirty-six pts were treated at diagnosis: 81% were at intermediate-high or high risk according to IPI and 44% had bone marrow involvement. Fourteen pts were in relapse/progression with advanced disease. Up to now, 41 pts are evaluable. An adequate PBPC yield (>5x10⁹/Kg) was obtained in 33/41 (median 11,9x10⁹/Kg CD34+). ASCT were not performed in 11 pts: 5 because of inadequate PBSC yield (2 in relapse and 3 heavily pre-treated) and 6 pts progressed during the therapy. For 6 pts the therapy is on going. 30/41 pts were autografted. The median times to achieve neutrophils >0.5x10⁹/L and platelets >50x10⁹/L were 6 days (range 4-9) and 16 days (range 2-79) respectively. Observed toxicities during the ASCT was as follows: 4 mucositis (grade 3), 3 gastrointestinal toxicity (grade 3-4), 5 infections (pneumonitis) and 1 neurotoxicity (grade 3). No toxic deaths occurred. Clinical response in the 27 pts treated as first line therapy was: CR 74%, PR 4% and NR 22%. Response in the 14 pts in relapse/progression was as follows: CR 57%, PR 7%, NR 36%. With a median follow-up of 26 months, 2-yr OS was 72% in pts ad diagnosis and 36% in pts relapse/progression; the relapse occurred in 4/20 in RC pts treated at diagnosis and in 5/8 in RC pts treated in relapse/progression respectively. Conclusions: these preliminary results show that an "age-adjusted" HDC regimen plus ASCT is feasible and effective with low toxicity also in elderly patients. A good PBS collection is achievable also in elderly pts after HDC with Mitoxantrone and HD-ARAC.

PU180
MANAGEMENT OF ON-LINE DATABASE FOR REGISTRATION OF AUTOLOGOUS STEM CELLS TRASPLANTATION AT THE HEMATOLOGY "LA SAPENZA" OF ROME. ACTIVITY UNTIL DECEMBER 2002

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Introduction: Since 1981 until December 2002, we performed 1,280 autotransplants in different hematologic malignant diseases. Since 2000 we were using the on-line registration program based on ProMise software, the same used for EBMT registration (European Group for Blood and Marrow Transplantation). Materia and methods: in this program a large number of data is available from diagnosis until last events. The data are periodically updated and can be used for many studies. Although the data are heterogeneous, with this program we are able to select and obtain an homogeneous population of patients according to the characteristics to analyse. Moreover, in a simple way we have a fast view on the activity and the use of autotransplant during the time. Results: with a series of simple specific queries, we obtain information about autotransplant activity in our institution, such as: 1) during the years the number of transplants has increased until 2000 with a reduction trend in last two years; 2) the percentages of autografts performed in the different diseases are: AM L 31.7%, ALL 6.3%, CML 8.7%, CLL 2.4%, MM 16.7%, NHL 26.1%, HD 7.6% and MDS 0.5%; but we have to take into account not only the absolute or relative number of transplants in the different diseases, but also how the indications change all over the time and the data clearly show the current indications to autograft in our institution; 3) in the last years the patients at transplant are older than some years ago; in fact before...
1991 only 5% of patients were older than 50 years, while in the last years the age at transplant has progressively increased and since 2001 more than 40% was older than 50 years; 4) currently transplant related mortality is 2% while in the '80s it was 10%. 5) PBSC are currently used in >90% of transplants and marrow cells are used only in particular cases. Conclusion: this on-line database offers the possibility to update in real time the data and follow-up, with a simple personal computer connected to internet. The use of this technology in medicine is a powerful instrument which allows to look at the data in a few time, but to give value to this it is necessary to update and check periodically the database.

PU91
RED BLOOD CELL TRANSPLANTATION IN PERIPHERAL BLOOD VS BONE MARROW TRANSPLANTATION. RETROSPECTIVE ANALYSIS ON 193 PATIENTS FROM A SINGLE INSTITUTION
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Background: Patients autografted with peripheral blood stem cells (PBSC) have a shorter duration of neutropenia and thrombocytopenia than patients who receive bone marrow cells. There is only little evidence in literature regarding the post-transplant erythroid reconstitution and the red blood cell (RBC) transfusion requirement in relation to the source of stem cells employed. Aims: To study whether the shorter duration of aplasia, due to the use of PBSC, can influence the RBC transfusion requirement, from a database of more than 1000 patients, we retrospectively selected a group of 193 patients (median age 34 years), who received transplants of either autologous bone marrow stem cells (BMSC) or PBSC for various hematologic malignancies. Methods: Patients were grouped homogeneously basing on diagnosis, disease status, type of chemotherapy regimen and type of stem cells reinfused, and for each group we analysed the engraftment and the RBC transfusion requirement in both, early and late post-transplant period. Results: In our analysis, significantly fewer transfusion of RBC were required utilizing PBSC. The significant reduction in RBC transfusion requirement was identical in both, number of patients requiring RBC transfusions and number of RBC transfusions required for each patient, in all samples of patients and in all intervals considered. Conclusions: In summary, our results demonstrated that the use of PBSC was superior to BMSC, not only with regard to engraftment, but also to RBC transfusion requirement, with possible additional advantage in terms of quality of life and cost-effectiveness.

PU92
AUTologous STEM CELL TRANSPLANTATION IS A SAFE AND EFFECTIVE PROCEDURE EVEN IN OLDER PATIENTS WITH MULTIPLE MYELOMA
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Autologous stem cell transplantation is actually considered the treatment of choice for newly diagnosed MM patients up to the age of 60 years. However, several lines of evidence suggest that also patients aged over 60 and with good performance status may be candidates for this procedure, although there is no current proof of a survival advantage for this age group in comparison with conventional chemotherapy. In the present analysis, we retrospectively identified 34 patients aged more than 60 years, of whom 17 were older than 65 years, who received an autologous transplantation of primed peripheral blood stem cells (PBSC) either as part of first-line therapy (n=30) or as salvage treatment following a prior autotransplant (n=4). Conventional treatments most frequently used for primary induction of remission were VAD or VAD-hybrids, whereas high-dose therapy (HDT) administered before autotransplant consisted of i.v. melphalan at 200 mg/m² in 31 patients or a combination of melphalan and busulfan in the remaining 2 patients. Following priming therapy with cyclophosphamide (CTX) at doses ranging from 4 to 7 g/m² according to patients’ age, the median number of collected CD 34+ cells was 7×10^6/kg, with no difference between the two age groups of patients who received lower or higher CTX dose. One patient failed to collect the minimum three-fold dose of 2×10^6 CD34+ cells. On an intent-to-treat basis, the overall rate of complete remission (at least or higher than partial remission) was 26% (82%); the corresponding figures for patients aged less than 65 years and higher than 65 years were 29% (82%) and 23% (82%), respectively. Both hematologic recovery and extramedullary toxicity were superimposable in both age groups. Overall, the frequency of grade 3-4 non-hematologic toxicity was 9%; a single patient died due to transplantation-related complications. With a median follow-up of 30 months, the 4-year projected probability of survival was 75% and median event-free survival was 38 months. It is concluded that age higher than 60-65 years did not adversely affect the outcome of patients receiving an autologous transplantation and, hence, should not constitute an exclusion criterion for participation in clinical trials with PBSC-supported HDT.

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ENDOCRINE DYSFUNCTION DURING THE FIRST YEAR AFTER STEM CELL TRANSPLANT FOR HEMATOLOGICAL MALIGNANCIES

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Aim of the study was to investigate the endocrine functions during the first 12 months after autologous SCT. The hypothalamic-pituitary-gonadal/thyroid/adrenal/GH-IGF-1 axis functions were investigated after 3 and 12 months in 82 patients (43M, 39F) aged 16-55 yrs (median, 39). All patients had received SCT for treatment of hematologic malignancies (AML, HD, LNH), 16 of them having previously received radiotherapy. Ninety% of women experienced secondary hypergonadotropic amenorrhea; menstrual cycles recovered in 15% of them and other 20% had improvement of FSH, LH and estradiol values during the follow up. In 86% of men, FSH values were above the normal range at first evaluation and improved thereafter in 30% of them. Low testosterone was found in 30% of men and improved with time in about half of them. Spermogram was performed in 15 men and revealed azo-oligospermia in all cases. Adrenal insufficiency occurred in 13 (15%) of patients, mostly treated by high-dose steroids before SCT for lymphomas. Subclinical hypothyroidism was found in 12% of patients, and improved in all but three patients who developed clinical hypothyroidism; all these patients had been treated by radiotherapy. Subclinical hyperthyroidism (TSH < 0.5 mIU/L, normal thyroid hormones) was found in 10.5% of patients, without anti-thyroid antibodies. Low T₃ syndrome was present in 8 patients (9%) and chronic thyroiditis with prevalent increase in anti-thyreoglobulin antibodies (50-630, median 162 UI/mL; normal < 40) was present in 17% of patients, all having normal thyroid function. Thyroid nodules were detected in 9 (10.5%) patients and were stable during the follow up period. IGF-1 values were below the normal range in 43% of patients and improved within the 12 th month, likely mirroring metabolic alteration following SCT rather than GH deficiency. In conclusion, endocrine abnormalities occur frequently during the first year after auto-SCT, and show a tendency to improve within one year. Antiblastic and radiation treatments were likely responsible for gonadal damage. Some kind of thyroid abnormality was found in 45% of patients, likely mirroring multiple factors, including previous treatments, abnormal immune system function and general health status. It remains to be established to which extent GH deficiency may be responsible for the IGF-1 decrease. Endocrinological follow up should be included in the follow-up patients treated by auto-SCT for hematologic malignancies.

THYMIC REBOUND AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Thymus is an immunologic gland that develops in embryonal stages showing its maximum of activity in the first years of life. Its function is to mature to and to educate T-lymphocytes. The visualization of the thymus and the frequent detection of volume increase, are typical of the pediatric age. In general, a residue of the gland persists in the young adult, but probably its function is negligible. Thus, in the adult imaging techniques do not allow visualization of thymic residues, and there are no codified conditions of increase of volume, unless rare cases of thymoma. Frequently, lymphomas (Hodgkin’s and non-Hodgkin’s) are localized in the mediastinum and not rarely in the antero-superior part of it. Almost always lymphomatous mass uptakes 67Ga and scintigraphy with 67Ga is often used as staging or restaging procedure. We observed 2 young adults presenting a mass in the thymic area with positive gallium uptake a few months after autologous transplant for lymphoma, with spontaneous regression in the following months. This phenomenon, called thymic rebound, may cause troubles in lymphoma restaging. A 18 y.o. w. affected by T-Lymphoblastic Lymphoma stage IV-A, was autotransplanted in first CR on May 1998; at month +4, she showed intense uptake of 67Ga in the anterosuperior mediastinal area, and CT scan showed a solid mass in the same region. At month +6 these imaging were unmodified. The patient refused any further investigation. Surprisingly, at month +9 CT scan and gallium scintigraphy were negative. On May 2003, CT scan and scintigraphy remained persistently negative. The second patient, a 17 y.o. m., affected by H.D. stage III-B, was autotransplanted in first CR; at month +5 he showed intense gallium uptake in anterosuperior mediastinal area, and a CT scan showed a solid mass in the same region. Tallium201 and PET performed at month +7 were negative. Thymic rebound is a rare condition that may occur a few months after intensive chemotherapy, especially in pediatric and adolescental age. Standard radiology and CT scan are not able to distinguish between relapse of lymphoma and thymic rebound; we were surprised by the intense positivity of 67Ga uptake in a benign hyperplasy. Negativity of 201-Tallium uptake seems to confirm the higher specificity of this radionuclide in recognizing neoplastic tissues. Even PET negativity may be helpful to perform a correct diagnosis of nature of masses in the thymic area without invasive methods. The finding of a single mass by CTscan and 67 Ga scintigraphy in
Background: Optimal high dose therapy (HDT) for autologous stem cell transplantation (ASCT) is yet to be established. In order to define the conditioning regimen with the greatest eradicative capacity and the lowest toxicity, we compared three different preparative regimens: melphalan 200 mg/m2 (MEL 200) in 44 patients, melphalan 200 mg/m2 plus carmustine 600 mg/m2 and vepeside 1200 mg/m2 (BEM) in 25 cases, and melphalan 200 mg/m2 plus thiotepa 10 mg/kg (TT-MEL) in 22 percent in the TT-Mel group. Regarding the MEL 200 group, compared to 56 percent in the BEM group, the incidence of treatment-related toxicity was lower (31 percent) than among those treated with TT-Mel (30 percent) or BEM (20 percent). Among patients with non responsive or progressive disease at ASCT, 33 percent of cases receiving MEL 200 entered CR, compared with 35 percent receiving BEM and 0 percent receiving TT-MEL. Conclusions: This study shows that the three preparative regimens for ASCT have similar efficacy with a trend for a lower treatment-related toxicity with melphalan alone. More intensive conditioning regimens, such as BEM or TT-MEL, do not improve the response and increase non-haematological toxicity.

PU195
A COMPARATIVE STUDY OF TOXICITY AND EFFICACY OF THREE PREPARATIVE REGIMENS FOR AUTOLOGOUS TRANSPANTATION IN MULTIPLE MYELOMA: MELPHALAN 200 MG/M² AS A SINGLE AGENT, OR ASSOCIATED WITH CARMUSTINE AND VEPESIDE, OR WITH THIOTEPA (A SINGLE CENTER STUDY)
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PU196
SEQUENTIAL HIGH DOSE CHEMOTHERAPY IN PATIENTS WITH RESISTANT OR RELAPSED LYMPHOMA: OUTCOMES AND PROGNOSTIC FACTORS
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From January 1992 and June 2001, 100 patients affected by resistant or relapsed lymphoma were enrolled in a high dose chemotherapy program (Cytoxan 7 g/m², Methotrexate 8 g/m², V P-16 2 g/m², mitoxantrone 60 mg/m²+Melphalan 160 mg/m² followed by PBSc autograft): 54 pts were affected by high grade non-Hodgkin lymphoma, 30 by Hodgkin's disease and 16 by follicular lymphoma. In this last group methotrexate treatment was omitted. All the pts started salvage treatment with a second line conventional chemotherapy (CHOP like or DHAP). At the transplant, 32 pts were in complete remission. Results. After autologous transplant complete remission was achieved in 97/100 patients. If we only consider the group of 68 patients with evidence of disease at the transplant, we obtained 65/68 CR (95%). Median follow up was 50 months. Overall survival (OS) and event free survival (EFS) by Kaplan-Meyer's was 50% and 46%, respectively at 5 years. It's to be noted that CR rate and EFS were independent from diagnosis. One patient died...
because of sepsis. Delayed complications. Secondary malignancies were observed in 6 patients: 2 cases of acute lymphoblastic leukemia, 2 cases of myelodysplastic syndrome, 1 adenocarcinoma of the lung and 1 case of breast carcinoma. All these patients died because of tumor. Congestive heart failure occurred within few months in 8 patients. All cases but one responded to treatment, and are at present on digoxin and diuretics with a good quality of life. One patient with dilated cardiomyopathy refractory to therapy, underwent cardiac transplantation. We considered the prognostic factors present at the time of relapse or at the time of patient enrollment in the program. In multivariate analysis we evaluated in the group of NHL: IPI score, B symptoms, LDH, ECOG, stage, the number of extra-nodal involvements and we found that LDH level, ECOG status and B symptoms maintained statistical significance for EFS. In the group of patients with Hodgkin disease, none of the analyzed factors (Hb level, LDH and albumin) showed any statistical prognostic significance.

Allogeneic Transplantation

PU197
NEW PREPARATIVE REGIMEN FOR BONE MARROW TRANSPLANTATION IN CLASS 3 YOUNG AND ADULT THALASSEMIC PATIENTS
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In April 97 we adopted a new Protocol for Class 3 thalassemic patients called Protocol 26. In this protocol we have the conditioning regimen starting on day - 45 from the transplant with hydroxyurea 30 mg/kg and azathioprine 3 mg/kg given daily until day -11, fludarabine 20 mg/m² given from day -17 to day -11. Both groups of the Class 3 patients, young and adults, received busulphan 14 mg/kg starting on day -10. Following the busulphan, in Class 3 patients aged less than 17 years, for whom the major problem was not the excess of toxicity, but the high rate of thalassemic relapse after the transplant, the total cyclophosphamide dose remained 160 mg/kg. In Class 3 patients older than 16 years, for whom the major problem was the toxicity, but not the return of the disease, the total dose of cyclophosphamide was reduced to 90 mg/kg. In the group of 31 Class 3 young thalassemic patients, two (7%), rejected the transplant and became thalassemic again and one (4%) died, with 97% of survival and 90% of thalassemia-free survival, at almost 5 years after the transplant. In the group of 14 adult Class 3 thalassemic patients, 4 died, two became thalassemic again, with 70% survival and 57% thalassemia-free survival.

PU198
IMATINIB MESYLATE (GLIVEC) PRECEDING ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PHILADELPHIA-POSITIVE ACUTE LYMPHOBlastic LEUKEMIA: A SINGLE CENTER EXPERIENCE
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Up to 20% of adults with acute lymphoblastic leukemia (ALL) express the Philadelphia chromosome (Ph) and its product, the BCR-ABL fusion protein. The prognosis of Ph+ ALL is extremely poor. Despite complete remission rates of 60-80% with conventional chemotherapy, the vast majority of patients suffer from an early relapse, and long-term survival is less than 10%. Allergic stem cell transplantation (SCT), when performed in first complete remission (CR), grants a long-term survival of 35-65%. Results of SCT are far lower when SCT is performed in second or subsequent CR6 or, even worse, in relapse. Timing is therefore crucial in the management of Ph+ ALL, and SCT should be carried out in the small window of the first CR usually attained with chemotherapy. Imatinib mesylate (Glivec) is a selective inhibitor of BCR-ABL tyrosine kinase, with a significant anti-leukemic activity in relapsed or refractory Ph+ ALL or chronic myeloid leukemia (CM) in lymphoid blast crisis, though response is usually short. Two recent reports have shown the safety and efficacy of Imatinib preceding allogenic SCT in relapsed or refractory Ph+ ALL and before allogeneic SCT and donor lymphocyte infusion (DLI) in Ph-positive acute leukemias (ALL and CML in lymphoid blast crisis). We report our experience with Imatinib therapy as a maintenance or salvage therapy preceding allogenic SCT in patients with Ph+ ALL. Between October 2001 and October 2002, four patients (3 males and one female) with Ph+ ALL were treated with Imatinib before allogenic SCT at our institution. The median age at diagnosis was 46 (range: 31-55) years. At cytogenetic analysis, all the 3 patients evaluable showed the classical t(9;22) translocation. The molecular study by RT-PCR was positive for P210 in all 4 patients (two b3a2 and two b2a2 transcripts); two patients co-expressed the P190 (e1a2) hybrid protein. The patients initially received two courses of chemotherapy (vincristine, doxorubicin, L-asparaginase and prednisone, then high-dose cytarabine and Idarubicin), then, if remission is achieved, two more courses of therapy with vincristine, methotrexate, cyclo-
phosphamid/phosphamid and cytarabine (or Adriamycin) were administered. Imatinib therapy was started when hematologic recovery after the fourth cycle was documented, or after the second course in the resistant patients. The daily dose of imatinib was 400 mg, then raised to 600 if tolerated. Three patients (75%) completed the four courses of chemotherapy: two obtained also a cytogenetic remission (i.e. 100% Ph-), one after the second course and the other after the fourth, but no molecular response was documented (one transient PCR-negativity was not confirmed). One patient was refractory to the first two courses. Imatinib therapy was given for a median of 120 days (range: 67-155) prior to allogeneic SCT. All the four patients maintained or attained the complete hematologic response, and two (50%) became BCR-ABL-negative (molecular response), after two months of therapy. Tolerance to imatinib was good in three patients, while the fourth developed grade III liver toxicity and reactivation of HBV infection which led to suspension of treatment. Two patients relapsed: one after suspension of imatinib therapy for liver toxicity, the second after 114 days of treatment. The first received intensive chemotherapy, attaining a second CR, and then proceeded to SCT; the latter directly underwent SCT. All SCT was performed in cytogenetic and molecular response in two patients, second CR in one and relapse in one patient. Three patients (75%) underwent transplantation from a matched unrelated donor (MUD), one from an HLA-identical sibling. Stem cell source was peripheral blood (PB) in 3/4 (75%) cases, bone marrow in 1/4 (25%). Conditioning regimen included cyclophosphamide (CY) and TBI in three patients, BUCY and ATG in one. GVHD prophylaxis consisted of cyclosporine and short-course methotrexate. All the three patients transplanted in remission engrafted, with a median time to neutrophil and platelet recovery of 17 (range: 13-29) and 22 (range: 17-23) days respectively. None of the patients developed an acute or chronic GVHD greater than grade I. The two patients who underwent the SCT in molecular remission, are actually in complete hematologic, cytogenetic and molecular remission after 15 and 5 months. The patients transplanted in second CR maintained a hematologic remission (but was always BCR-ABL-positive) for 3 months, and then relapsed again; he is actually in third CR after DLI, 8.5 months after SCT. The patients transplanted in relapse, developed a grade III-IV acute GVHD (skin, liver, bowel) and died on day +27 after SCT. Our experience confirms the safety and efficacy of imatinib therapy preceding allogenic SCT recently reported.10,11 Imatinib is effective in maintaining a remission attained with intensive chemotherapy, and can improve the degree of this response to the molecular level therefore reducing the bulk of disease to the minimum before allogenic SCT. Imatinib therapy does not seem to affect the transplant procedure in terms of engraftment or GVHD.

References
cell transplantation (APBSCT) but he relapsed after 2 months. He underwent a non-myeloablative stem cell transplant from an HLA ID Sib. Before transplant the skin lesions were stable. The patient was conditioned with Fludarabine and M elphalan. He received 8.5×10^6 CD34/kg of recipient body weight, aGVHD prophylaxis with Cyclosporin 3 mg/kg in 2 divided doses from day -1 and MMF 15 mg/kg twice daily from day 0. The patient was discharged to the outpatient department on day +3, remained apyretic throughout transplant and no mucositis or VOD was recorded. Weekly clinical observation were performed to follow the skin lesions. Bone marrow chimeraism showed full donor by day +28. M M F was discontinued on day +27 and P DN was tapered quickly, without flare of GVHD. On day +45 clinical evaluation showed a fast increase of the skin nodule. On day +5 the patient had skin aGVHD grade II flare and nausea and loss of appetite, not responsive to prednisone and in a small amount of days developed a florid, fatal, hemophagocytic syndrome. We conclude that a non-myeloablative transplant for this patient was well tolerated; it was possible to induce a full donor chimera by day +28 but the fatal evolution can reduce the role of a non-myeloablative transplant in the setting of this disease where immunosuppression is required to induce a mixed chimerism to be used as a platform for adoptive immunotherapy.

**PU200**

**RECOVERY OF DENDRITIC CELLS (DC1 AND DC2) AFTER ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTATION**


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In peripheral blood (PB) two subsets of dendritic cells (DC) have been identified: DC 1, myeloid, are lineage negative, HLA-DR positive and CD11c positive; DC 2, lymphoid, are lineage negative, HLA-DR positive and CD123 positive. DC1 activate T lymphocytes, while DC2 activate T lymphoid, are lineage negative, HLA-DR positive. DC1 and DC2 have been identified: DC 1, myeloid, are lineage negative, HLA-DR positive, CD11c positive (DC1) or CD123 positive (DC2). Prior to the beginning of conditioning regimen the mean number of DC1 and DC2 (per microliter) was 2.9±0.1 and 3.1±0.9 (group 1), 2.0±0.5 and 2.5±0.1 (group 2) and 2.1±0.6 and 2.4±0.5 (group 3): these numbers were similar to those of autologous patients (2.9±0.1 and 2.8±0.1), but lower than in normal donors (6.0±1.0 and 6.7±0.1, respectively). In autologous patients at day 0 and day +7 the mean DC1 and DC2 number was lower than 0.02±0.001 while in allogeneic patients DC1 and DC2 could be not detected. All autologous patients recovered to the pre-transplant number of DC1 and DC2 within day plus 20 from transplant (3.0±0.1 and 2.7±0.1), reaching to normal numbers after 6 months from transplant (6.2±0.1 and 5.7±0.1). In allogeneic patients instead, pre-transplant values of both DC1 and DC2 were reached at day +90 in group 1 (2.7±1 and 3.5±1.8), at day +180 in group 2 (3±1.6 and 3.1±1.7) and at day +270 in group 3 (4.5±1.9 and 2.1±0.1). One year after transplant mean number of DC1 and DC2 was still lower than in normal subjects in every group (3.5±1.1 and 2.5±0.1; 3.5±1 and 3.7±1.5; 3.7±2 and 2.0±1.1). The delay of recovery at pre-transplant level in group 2 and 3 could be related to the major incidence of acute and chronic GVHD in these groups than in selected patients. On this basis, we observed that the absolute number of both DC1 and DC2 was lower in patients who developed acute and chronic GVHD than that in the other patients. Moreover we observed that in patients who developed acute GVHD the ratio between DC1 and DC2 was always more than 1. In conclusion, DC1 and DC2 recovery is markedly delayed following allogeneic transplant if compared with autologous transplant: this is probably linked to a delay in immune system reconstitution and to the onset of acute and chronic GVHD.
was 4.9 $\times 10^6$ /kg donor weight (range, 1.3-22.3) in the *Institute of Haematology, University of Perugia, Italy; Aversa F,* Falini B* 20) CD34+ median number of 9.4 (range 6-17,4) and 10 (range 0-20) CD34+ cells was reached in all cases with a median number of 9.4 (range 6-17.4) and 10 (range 0-20) CD34+ $\times 10^6$/kg donor weight collected, respectively (p=ns), and a median of 2 procedures (range 1-4) performed in both groups. SCC was associated with a decrease in platelet count, which was not significantly different between two groups; platelet count was within normal limits 5.5±3.4 and 8.7±6.1 days from last aphaeresis, respectively (p= 0.016) while WBC returning to baseline values at mean days 9.4±8 in group I, and 9.5±7 in group II (p=ns). In conclusion lenogastim and filgrastim mobilization of PBPC were similarly in terms of collection and short-term and long-term adverse effects in HD.

PU202
HAPLOIDENTICAL PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR ALK-POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA
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Anaplastic large cell lymphoma (ALCL) expressing anaplastic lymphoma kinase (ALK) as either the NPM-ALK fusion protein or alternative ALK-containing fusion proteins (for example, TPM3-ALK, TRK-ALK, Clathrin-ALK, ATIC-ALK, and Mosin-ALK) is a rare disease exhibiting distinctive molecular, pathological and clinical features. It usually occurs during the first three decades of life and presents as disseminated (stage III-IV) disease with systemic symptoms. Although many patients can be cured by chemotherapy alone, chemoresistance is associated with poor prognosis. Here, we report the clinical and pathologic characteristics of a patient with chemoresistant ALK-positive ALCL and its successful treatment with haploidentical peripheral blood stem cell transplantation (PBSCT). Report of cases such as that described in this work is important since ALK-positive ALCL is often confused with other lymphoma subtypes and even misdiagnosed as a reactive condition. The present case shows that haploidentical PBSCT transplantation should be offered, as last option, to selected patients with chemoresistant ALK-positive ALCL.

PU203
NEUROLOGIC COMPLICATIONS FOLLOWING AUTOLOGOUS AND ALLOGENEIC BONE MARROW TRANSPLANTATION. THE EXPERIENCE OF A SINGLE INSTITUTION
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Stem cell transplantation (SCT) is associated with a spectrum of neurologic complications (NC) secondary to the underlying disease, prolonged myelosuppression and the use of immunosuppressive drugs. The incidence and variety of NC are related to the type of SCT and are mostly due to infection, vascular events, hemorrhage, drug toxicity and metabolic encephalopathy. In this study, we retrospectively analyzed severe NC in consecutive patients who underwent SCT to compare the frequency and type of these complications between patients who had allogeneic SCT (Allo-SCT) or autologous SCT (Auto-SCT), and to ascertain what factors may predispose to particular NC. A total of 740 SCTs (Auto-SCTs: 153 (20.6%) were performed from February 1992 to May 2003. Overall, we observed 20 episodes (2.7%) of severe NC in 19 patients. Fourteen out of 19 cases underwent Allo-SCT (2 with haploidentical donor), utilizing as the source of stem cell marrow (6), peripheral blood (7) and cord blood (1). Six underwent autologous (Auto)-SCT, 5 received peripheral blood and 1 marrow cells. Twelve were male and 7 female, with a median age 45 years (range 5-64 yrs). The underlying diseases were acute leukemia (9), myeloproliferative disorders (6), lymphoproliferative disorders (4) and metastatic breast cancer (1). All patients were uniformly treated with infectious, thrombosis, and hemorrhagic prophylaxis. Moreover, the patients at high risk of drug-induced neurotoxic effects were empirically treated with phenobarbital. Patients were conditioned with busulfan- (9), melphalan- (7), fludarabine- (2) and TBI-based (2) regimens. Twelve of the 20 cases that experienced NC were heavily pre-treated at the time of SCT. According to Krouwer et al. (Neur Clin 21, 2003), in the setting of Allo-SCT we classified 7 episodes with M RI- defined as metabolic encephalopa-
thy (2) and drug induced (5). Seven cases had focal neurological deficits and M Ri+: 5 vascular events (2 TTP, 1 TVP and 2 vasculitis), 1 fungal infection with multiple brain abscesses and 1 Wernicke's syndrome, responding to high doses of thiamine. Out of the 5 severe NC associated with Auto-SCT, one patient developed drug toxicity two days after beginning the conditioning regimen; 4 patients showed focal neurological and radiological abnormalities: 2 cases due to cerebrovascular events (1 infarction and 1 hemorrhage) and 2 likely due to infectious disease. The median time to the onset of neurological symptoms was 49 days (range 18-97 days) for Allo-SCT and 15 days (range 7-160 days) for Auto-SCT. NC-related mortality was 42.8% and 60% in Allo-SCT and Auto-SCT, respectively. In conclusion, the difference frequency and type of neurologic complications between allogeneic and autologous BMT mostly reflected the different degrees of treatment related morbidity and immunosuppression of both types of SCT.

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PU205
MINOR HISTOCOMPATIBILITY ANTIGENS AND ACUTE GRAFT VERSUS HOST DISEASE (aGVHD) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEREDITARY HEMATOLOGICAL DISEASES
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It has been suggested that donor/recipient incompatibility for non-HLA antigens (minor histocompatibility antigens-mHA) are responsible for aGVHD after hematopoietic stem cell transplantation (HSCT) from HLA-identical donors. Clinical studies in patients with hematological malignancies confirmed this hypothesis and showed that the polymorphic antigens H-Y, HA-1, CD31-125, CD31-563, and some HPA play the role of aGVHD, in that mismatch at these antigens increased the risk of aGVHD. In particular, in a case series of 150 consecutive patients (100 children and 50 adults), we recently showed that incompatibility at CD31-563 was significantly associated with a high risk of aGVHD (OR 3.97, P 0.008). So far, no study examined the effect of mismatch at these antigens in patients receiving HSCT for non-neoplastic illnesses. The aim of the present research was to investigate the role of mismatch at H-Y, HA-1, CD31-125, CD31-563, HPA-1, HPA-2, HPA-3 and HPA-5 in the occurrence of aGVHD in patients receiving HLA-identical HSCT for inherited hematological disorders. 149 patients (121 children and 28 adults) were enrolled in the study. 132 patients received HSCT for thalassemia, 11 for sickle-cell anemia, and 6 for Diamond-Blackfan anemia. 135 patients received HSCT from a sibling, 2 from a parent and 12 from an unrelated donor. GVHD prophylaxis consisted of cyclosporin A as short course of methotrexate. Characterization of HPA-1, HPA-2, HPA-3, HPA-5, the polymorphisms at codon 125 (leucine/valine) and 563 (serine/asparagine) of CD31, and HA-1 antigen has been performed by genomic typing. Donor/recipient pairs were considered to be incompatible at the HPA, HA-1, CD31-125 and CD31-563 when the recipient had an allele foreign to the donor. Pairs were defined incompatible at the male spe-

PU204
HIGH DOSE ERYTHROPOIETIN α IN PATIENTS WITH PERSISTENT ANEMIA AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION
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Background. The use of erythropoietin α (EPO) at the conventional dose of 10,000 IU three times a week is often ineffective in the treatment of non-hemolytic and non hemorrhagic anemia after allogeneic bone marrow transplantation (BMT). Patients and methods. We tested the use of high dose of EPO after BMT in 18 pts, grafted for CGL8, ALL1, AM7, HD1, MDS1 from HLA identical sibling donor, HLA mismatched family donor and HLA matched unrelated donor. The conditioning regimen was with TBI in 9 pts. EPO was administered in 15pts with hemoglobin level <9g/dL and in 3pts with hemoglobin <10g/dL. Patients were treated with EPO at the dose of 40,000 u. i. twice a week for two consecutive weeks, then 40,000 u. i. weekly for two weeks and then 40,000 u. i. twice a month for 1 month. Seven pts (39%) were transfusion dependent at the beginning of the treatment. The interval between BMT and EPO treatment was 104 days (range 45-300). Results. The increase of Hb level was 2.3g/dL at 15 days (range 1-4), 3.6 g/dL at 30 days (range 2-7) and 1.7 g/dL at 60 days (range 1-4); there was no response in 2 pts: one relapsed after BMT and the other one developed persistent CMV infection. No decrease in WBC and PLT count has been observed; no other side effect. All the pts became transfusion independent. Conclusions. The use of EPO after BMT improves Hb levels, reduces transfusion requirements and improves quality of life.

PU206
CELL TRANSPLANTATION FOR HEREDITARY HEMATOLOGICAL DISEASE (AGVHD) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEREDITARY HEMATOLOGICAL DIS
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research was to investigate the role of mismatch at H-Y, HA-1, CD31-125, CD31-563, HPA-1, HPA-2, HPA-3 and HPA-5 in the occurrence of aGVHD in patients receiving HLA-identical HSCT for inherited hematological disorders. 149 patients (121 children and 28 adults) were enrolled in the study. 132 patients received HSCT for thalassemia, 11 for sickle-cell anemia, and 6 for Diamond-Blackfan anemia. 135 patients received HSCT from a sibling, 2 from a parent and 12 from an unrelated donor. GVHD prophylaxis consisted of cyclosporin A as short course of methotrexate. Characterization of HPA-1, HPA-2, HPA-3, HPA-5, the polymorphisms at codon 125 (leucine/valine) and 563 (serine/asparagine) of CD31, and HA-1 antigen has been performed by genomic typing. Donor/recipient pairs were considered to be incompatible at the HPA, HA-1, CD31-125 and CD31-563 when the recipient had an allele foreign to the donor. Pairs were defined incompatible at the male spe-

PU205
MINOR HISTOCOMPATIBILITY ANTIGENS AND ACUTE GRAFT VERSUS HOST DISEASE (aGVHD) AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HEREDITARY HEMATOLOGICAL DISEASES
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It has been suggested that donor/recipient incompatibility for non-HLA antigens (minor histocompatibility antigens-mHA) are responsible for aGVHD after hematopoietic stem cell transplantation (HSCT) from HLA-identical donors. Clinical studies in patients with hematological malignancies confirmed this hypothesis and showed that the polymorphic antigens H-Y, HA-1, CD31-125, CD31-563, and some HPA play the role of aGVHD, in that mismatch at these antigens increased the risk of aGVHD. In particular, in a case series of 150 consecutive patients (100 children and 50 adults), we recently showed that incompatibility at CD31-563 was significantly associated with a high risk of aGVHD (OR 3.97, P 0.008). So far, no study examined the effect of mismatch at these antigens in patients receiving HSCT for non-neoplastic illnesses. The aim of the present research was to investigate the role of mismatch at H-Y, HA-1, CD31-125, CD31-563, HPA-1, HPA-2, HPA-3 and HPA-5 in the occurrence of aGVHD in patients receiving HLA-identical HSCT for inherited hematological disorders. 149 patients (121 children and 28 adults) were enrolled in the study. 132 patients received HSCT for thalassemia, 11 for sickle-cell anemia, and 6 for Diamond-Blackfan anemia. 135 patients received HSCT from a sibling, 2 from a parent and 12 from an unrelated donor. GVHD prophylaxis consisted of cyclosporin A as short course of methotrexate. Characterization of HPA-1, HPA-2, HPA-3, HPA-5, the polymorphisms at codon 125 (leucine/valine) and 563 (serine/asparagine) of CD31, and HA-1 antigen has been performed by genomic typing. Donor/recipient pairs were considered to be incompatible at the HPA, HA-1, CD31-125 and CD31-563 when the recipient had an allele foreign to the donor. Pairs were defined incompatible at the male spe-
specific H-Y antigen when a male received a BMT from a female donor. Molecular typing showed that 13.2% of donor/recipient pairs were incompatible for HPA-1, 15.8% for HPA-2, 23.1% for HPA-3, 13.2% for HPA-5, 26.7% for CD31-125, 23.8% for CD31-563, 20.5% for HA-1 and 22% for H-Y. Only 16 out of 151 patients (10.6%) experienced grade II-IV aGVHD. No statistical correlation between the occurrence of aGVHD and donor/recipient incompatibility for each of the investigated antigens was identified. Also the number of mismatches did not influence the frequency of aGVHD. In conclusion, aGVHD is a rare event after allogeneic HSCT for inherited hematological disorders and, at variance with hematological malignancies, the incompatibility at the investigated polymorphisms has no clinical relevance.

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ICE APPLICATION IN MOUTH DURING METHOTREXATE ADMINISTRATION FOR PREVENTING MUCOSITIS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION


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 PU206

Short term methotrexate during CsA administration is the standard GVHD prophylaxis in stem cell transplantation. We prospectively evaluated 36 patients who underwent stem cell transplantation for hematologic malignancies. Previous reports showed the application on the mucous membrane of the oral cavity was the only measure, according to Cochrane review, of proven efficacy as a prophylactic treatment. The review found good evidence in two randomised trials that it halved the risk mucositis (relative risk 0.57, 95% CI 0.43-0.77). We first evaluated the timing of the ice application in the mouth by a curve of methotrexatemia in 12 patients every 30 minutes for 2 hours and half after the i.v. infusion of MTX for each methotrexate dose given. In this way we observed that the slope of the curves was similar and the time point of the best application could be during all administration of MTX until the 60’ minute after the end. The median age of the patients was 37.5 (16-61); 24 (66.6%) were males and 12 (33.3%) females. 18 patients (50%) received bone marrow, 17 (47.2%) peripheral blood stem cell and one (2.4%) cord blood as source of stem cell. 15 patients received reduced intensity conditioning; the therapeutic conditioning regimens were TBI based in 7/36 patients and Busulfan based in 14/36 patients. 70% of transplants were from a sibling and 30% from a VUD donor. The evaluation of the severity of the mucositis was determined by a WHO scale and date were collected with an Institutional form by the nurse staff. Also the duration of the mucositis and the requirement of the analgic therapy were recorded. Among such a study population, the incidence of mucositis grade III was 23.8% after standard conditioning versus 0% in those who received reduced intensity and grade IV was 19% after standard conditioning versus 6.6% in patients receiving reduced intensity. The median duration of mucositis was 14 (7-29) days for patients receiving standard conditioning regimens versus the 8 (6-26) days in who receiving reduced intensity regimens. Even if it was a phase II study, and no formal comparisons are allowed, we tried to evaluate if the historical data from patients transplanted in the same Institution and nursed by the same staff could suggest some advantages from this simple physical treatment. The incidence of grade III-IV mucositis in the historical control was 64.7% independently of conditioning regimens versus 27.8% of patients treated with ice during MTX. Among patients who underwent standard conditioning for their transplant the duration of mucositis was 19 (9-39) and 14 (7-29) days in the historical and in prospective group, respectively. These data suggest that ice application in the mouth could be an easy strategy capable of improving the grade III-IV mucositis. A multicenter prospective randomized study will be planned in order to evaluate the effectiveness of this procedure.

LOW DOSE RABBIT ANTITHYMCYTE GLOBULIN PREVENTS GRAFT-VERSUS-HOST DISEASE IN SIBLING ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA


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Our initial experience (November 1995 from to September 1999) with peripheral blood stem cell (PBSC) transplants in 21 patients with chronic myeloid leukemia (CML) resulted in an unacceptable rate of extensive chronic GVHD. For this reason, starting from October 1999 we have added low dose rabbit ATG in the conditioning regimen. We present here a comparison between the two groups. A total of 31 allogeneic peripheral blood stem cell transplants (SCT) from HLA identical sibling donor for CM L were performed. Median age was 41 years (range 19-53), 21 males and 10
females. The median interval diagnosis-transplant was 14 months (range 4-59). Conditioning treatment consisted mainly of BU-CY and the GVHD prophylaxis was short term MTX+CsA. 10 CML patients received low dose rabbit ATG (15 mg/kg, Fresenius). All patients engrafted and no rejection occurred. Acute and chronic GVHD were graded according to the standard criteria in patients surviving longer than 30 and 90 days after allogeneic SCT. Grade II-IV acute GVHD developed in 7/21 patients who didn’t received ATG and 2/10 patients who received ATG. Chronic GVHD developed in 15/21 patients without ATG and in 4/10 patients with ATG. In particular we observed 2 extensive cGVHD in the latter cohort (10 patients) and 10 in the former one (21 patients). Five hematological relapses occurred in the no-ATG group (2 pts CP transplants 3 pts in advanced phases) and no relapses in the other group. The patients were strictly studied at molecular level for the bcr-abl rearrangement after transplants; the incidence of isolated positivity was similar in the two groups. In conclusion, even if the number of patients is small and this was not a randomized study, we suggest that the use of low dose rabbit ATG is effective in decreasing the rate of GVHD, in particular extensive chronic GVHD, without increasing the risk of relapse.

PU208 GLIVEC FOR THE TREATMENT OF RELAPSE AFTER ALLOGENEIC TRANSPLANTATION IN CHRONIC MYELOID LEUKEMIA: EVALUATION OF MOLECULAR RESPONSE AND TOXICITY IN SIX PATIENTS
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From 2000 to January 2003, six patients relapsed after allogeneic stem cell transplantation. All of them were treated with Imatinib mesylate for relapse. Five out of six were transplanted from sibling and one from a VUD donor. Median age was 46 years ranging from 29 to 65 years. Median interval between transplant and relapse was 4 years (range: 14 months-11 years). Five patients were transplanted in chronic phase and all relapsed in chronic phase except for one. Two of the 4 CP-relapse were cytogenetic relapses. The patient transplanted in AP relapsed after 11 years from BM TRM of 9.5 and 42% at two years. For 1st CR patients transplanted in 1st CR was 69% at 5 years whereas the probability of survival was 21.5% in high risk patients. Similarly, relapse probability at 2 years was 9.5 and 46% respectively. Finally also TRM was dependent on the disease phase at transplant, with a TRM of 9.5 and 42% at two years. For 1st CR patients
death causes were disease in 2, GVHD in 6 and MOF in 2; for high risk patients disease in 9, GVHD in 3 and MOF in 4, intracranial hemorrhage in 1, and myocardial infarction in 1. In conclusion, in our experience the disease phase was strongly associated with outcome of allogeneic stem cell transplantation.

**PU110**

**CLINICAL AND PROGNOSTIC EVALUATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE AFTER SIBLING ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION**


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From 1995 to 2001 104 allogeneic peripheral blood stem cell transplants (SCT) have been performed from a sibling HLA fully matched donor with a myeloablative conditioning regimen. All the patients received the same GVHD prophylaxis with CsA and short term methotrexate (15-10-10-10 mg/m²). Median age was 41 years (range 19-57), 76 were males and 28 females. The diagnoses were acute leukemia or myelodysplastic syndrome in 43 patients, chronic myeloid leukemia in 20 pts, multiple myeloma in 32, lymphoma in 8, myelofibrosis in 1. Conditioning treatment consisted mainly of BU-CY (70) or unfractioned single-dose TBI (34). 10 CM L patients received low dose rabbit ATG (15 mg/kg, Fresenius). All patients engrafted and no rejection occurred. Acute and chronic GVHD were graded according to the standard criteria in patients surviving longer than 21 and 90 days respectively. 31 patients (29.8%) experienced acute GVHD gr II-IV and 16 (15.4%) gr III-IV acute GVHD. In patients surviving more than 90 days, chronic GVHD occurred in 52/94 evaluable patients; in particular in 32/52 it was extensive. The median number of CD34⁺ cells infused was 6.3×10⁹/kg (range: 0.9-19.5×10⁹/kg). No effect of the number of CD34⁺ was observed both on survival and on cGVHD occurrence. The results from a multivariate analysis on chronic GVHD occurrence will be given in details. Briefly, sex mismatch, age (>41 years) and a prior gr. II-IV aGVHD were retained in the final model of the multivariate analysis. The clinical characteristics of chronic GVHD from PBSC transplant were also analysed in term of the topography of the organ involvement, duration of the immunosuppressive therapy, withdrawal probability of immunosuppression, relationship with tapering of CsA, response to therapy and impact on survival. Extensive chronic GVHD became the main cause of death, appeared independently from CsA tapering and resulted quite resistant to multiple courses of therapy.

**PU111**

**NEUROLOGICAL COMPLICATIONS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE STUDY OF PROGNOSTIC FACTORS IN 183 PATIENTS**

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Objectives. To assess the clinical and etiological features of neurological complications (NC) of bone marrow transplantation (BMT). Materials and Methods. Review of clinical records of patients who underwent allogeneic BMT during the years 1997-2001 at the Seràgnoli Institute, Bologna. Results. NC were found in 36% of patients (66/183), usually involving the central nervous system (CNS). Most NC appeared within 6 month after BMT. The most frequent disturbances of CNS were tremor, and seizures, whereas polynuereopathy and myopathy were the main complications of the peripheral nervous system. The etiology was multifactorial, cyclosporin toxicity and CNS infection being the main causes. Cerebrovascular complications were relatively few (2 patients) in our series. Neurotoxicity of the conditioning regimen also played an important role. Thirty-three (50%) patients with NC died at 1 year. Discussion. NCs after BMT were frequent and the CNS was mainly involved. NC included a variety of manifestations, including major neurological features. A high mortality was detected in BMT recipients suffering with NC. Immunosuppression toxicity and CNS infections were the main cause. Conclusions. In univariate analysis the type of the transplant (VUD vs sibling) and busulfan-based regimens were significantly associated with NC within 6 months of BMT, whereas in multivariate analysis only the former resulted statistically significant.

**PU112**

**INDUCTION OF NK AND LAK ACTIVITY BY HUMAN CD34⁺ BLOOD CELLS**


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It has been previously demonstrated that purified CD34⁺ cells may induce allogeneic T cell proliferation and generation of cytotoxic. In this study, we addressed
the hypothesis of whether CD34+ cells may activate also NK cells. A standard 51Cr- assay was used to determine the ability of mnc from healthy donors to lyse HLA-negative, NK-sensitive (K562 cells) target cells, before and after culture with irradiated purified CD34+ cells or purified CD14+ monocytes, as control antigen-presenting cells (APCs), from allogeneic G-CSF mobilised donors. Stimulation by CD34+ cells or monocytes resulted in an increased ability of allogeneic mnc to lyse K562 cells (26±14% before MLC and 49±26% and 40±13%, respectively, after culture, at a 10:1 E/T ratio (n=3). However, while the generation of monocyte-induced cytotoxic activity was prevented by costimulatory blockade (with CTLA4-Ig at 2 μg/mL) by 53±18% (n=3), CD34+ cell-induced cytotoxic activity was only slightly affected (by 21±15%, n=3). Stimulation by CD34+ cells was associated with the recovery of greater numbers of CD3-CD56+ NK cells on average as compared to monocytes (85±19% vs 36±21%) (n=3). Depletion of CD4+ helper T cells in the responder population did not prevent CD34+ cell- and monocyte-induced K-562-targeted cytotoxic activity (76% and 42%, at a 1:10 E/T ratio, respectively). We then tested whether coculture of purified NK cells with CD34+ cells would result in the lysis of NK-resistant Daudi cells (LAK activity). While LAK activity was negligible before culture, it substantially increased upon culture with CD34+ cells, but not monocytes (to 51±15% and 25±14%, respectively) (n=3); addition of high dose IL-2 (at 1000 U/mL) was maximally effective, as induced a lysis of 67±12% at a E/T ratio of 10:1 (n=3). Finally, NK cells cultured with CD34+ cells produced greater amounts of IFN-γ and TNF-α (500 pg/mL and 125 pg/mL vs 50 pg/mL and less than 5 pg/mL) as compared to monocytes, although both cytokines were produced at a log greater amount upon culture with IL-2. Therefore, our data show that CD34+ cells directly activate NK cells in vitro. These results may suggest that interactions between donor CD34+ cells and host NK cells could play a role in graft rejection. Alternatively, they might be important in graft-versus-leukemia reactions following HLA-mismatched allogeneic hematopoietic stem cell transplantation.

Plasmacytoid DC (pDC) are a newly discovered subset of human DC that, upon viral or bacterial stimulation, may produce high amounts of IFNα and IFNβ; and induce either Th1 or Th2 antigen-specific T cells. Moreover, there is initial evidence suggesting that pDC may contribute to graft-versus-host reactions following allogeneic hematopoietic stem cell transplantation as well as to autoimmune diseases. In this study we tested whether pDC function and survival is affected by corticosteroids, a class of immunosuppressive agents widely used in the treatment of GVHD and autoimmune diseases. pDC were immunomagnetically isolated from the peripheral blood of healthy donors by using microbead-conjugated anti-BDCA4 antibodies (Miltenyi Biotec, Auburn, CA) and cultured for 48 hours with or without hydrocortisone (HC, 10-4 through 10-6 M) in the presence of bacterial CpG DNA. While it is known that viability of pDC decreases rapidly upon in vitro culture, CpG consistently allowed >50% pDC recovery at 48 hours (n=3 exps). Addition of HC consistently inhibited pDC recovery to less than 30%. Moreover, CpG-cultured pDC upregulated expression of HLA molecules and costimulatory molecules CD80, CD86 and CD40 (84%, 53% and 91% in 1 out of 3 representative experiments), as well as of maturation markers, such as CD83 (72%), consistent with their terminal differentiation to fully competent APCs. Maturation of pDC was inhibited by HC, as shown by decreased levels of CD80, CD86, CD40 and CD83 (60%, 25%, 70% and 34%) (n=3 experiments). To test whether pDC migratory properties were also affected by HC, we analysed the expression of the lymph-node homing molecules CCR7 and CD62L on pDC surface. CpG-cultured pDC upregulated CCR7 (from 14% to 74%, n=3), while downregulating CD62L (from 70% to 40%, n=3), whether or not HC was present, suggesting that pDC migration is not affected by corticosteroids. To study T cell activation by pDC, purified allogeneic CD4+ T cells were cultured with pDC, then production of T-cell derived cytokines was tested. Production of IFNγ, IL-10 and IL-2 by pDC-activated T cells was reduced by 5-10 fold when HC-exposed pDC were used as stimulators (IFNγ x2.4 vs 0.5 ng/mL; IL-10 0.1 vs 0.02 ng/mL and IL-2: 0.056 vs 0.007 ng/mL, 1 out of 3 experiments), whereas IL-5 was not detected in either condition. Finally, to test whether HC affected the ability of pDC to induce accumulation of other inflammatory cells, we measured CpG-induced pDC production of various chemokines. Interestingly, HC strongly inhibited the production of certain T-cell attracting chemokines, such as MIP3b (280 vs 17 pg/mL, respectively, without or with HC, n=2 experiments), whereas production of neutrophil-attracting, C-C like chemokines, such as GRO-β, was increased (16 vs 128 ng/mL). In conclusion our data suggest that corticosteroids may selectively block the initiation of T-cell responses by pDC, by reducing their ability to attract as well as stim-
ulate antigen-specific T lymphocytes. Conversely, survival and migration of pDC, as well as their participation in innate responses, are not affected by corticosteroids, as shown recently for production of IFN-\(\gamma\). Further studies are needed to assess whether this observation is relevant to the immune suppressive effects of corticosteroids in vivo.

**PU214**
CORTICOSTEROIDS PREVENT MATURATION OF MONOCYTE-DERIVED AND PERIPHERAL BLOOD CIRCULATING MYELOID DENDRITIC CELLS
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Myeloid dendritic cells (mDC) have been shown to play an essential role in the initiation of pathological immune responses such as graft-versus-host disease. In this study we investigated whether corticosteroid (CS) treatment may prevent their generation and/or function. mDC in tissues may originate from at least two different pathways: under steady state conditions, low numbers of peripheral blood circulating immature DC continuously migrate to peripheral tissues, whereas during inflammation large numbers of mDC may originate from circulating CD14+ monocytes. Immature circulating mDC were immunomagnetically purified as CD19-BDCA1+, while monocyte-derived DC were generated by culturing immunomagnetically purified CD14+ monocytes for 6-8 days with GM-CSF (50 ng/mL) and IL-4 (800 U/mL). Cultures were performed in the presence or absence of Hydrocortisone (10-6 M). Differentiation of mono-DC, defined as CD1a+ CD14- cells, was completed blocked by HC (0.7±1% CD1a+CD14- and 63±22% CD14+ cells after culture with HC as compared to 64±28% and 3.5±3%, respectively, without) (n=6). M icroscopic examination of cytospins confirmed that culture with HC prevented the appearance of cells with DC morphology. Moreover, HC prevented further maturation of immature monocyte-derived DC, as shown by the decreased expression of both BDG0 (71±10% vs 86±3%) and CD86 (45±22 vs 90±6%), with HC as compared to control, following culture with Lypopolysaccaride (LPS) (n=7 exps). Maturation of freshly isolated PB mDC was similarly affected by HC (n=2). HC-cultured DC, either mono-derived (n=3) or freshly isolated from PB (n=3), were weaker stimulators of allogeneic CD4+ T cells, by 70% and 50%, respectively, as compared to control-cultured DC, at all stimulator-responder ratios. Moreover, production of both IFN\(\gamma\) and IL-5 by DC-stimulated CD4+ T cells was inhibited by >90% when maturation of DC had occurred in the presence of HC (n=3), suggesting that HC-cultured DC are unable to induce both Th1 and Th2 responses. Similar effects were observed in CD4+ T cells activated by mature PB circulating mDC, although production of IL-5 was more affected (45±13% reduction) than IFN\(\gamma\) (only 18±5%) (n=3). In conclusion, our data suggest that treatment with corticosteroids may effectively target mDC, by inhibiting maturation of circulating immature DC as well as by preventing generation of new mDC from circulating monocytes. These data may prompt future clinical studies targeting host mDC to prevent aGVHD.

**References**

**PU215**
ALLOGENEIC GRAFT CD34+ CELL DOSE CORRELATES WITH DENDRITIC CELL DOSE AND CLINICAL OUTCOME, BUT NOT WITH DENDRITIC CELL RECONSTITUTION AFTER TRANSPLANTATION
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Previous studies have suggested that high doses of CD34+ cells in allogeneic hematopoietic grafts may facilitate the occurrence of acute or chronic GVHD, possibly because of the expansion of donor antigen-presenting cells. In this study we examined whether the CD34+ cell dose in PBSC or marrow allografts correlates with the dose of myeloid dendritic cells (mDC) and plasmacytoid DC (pDC), and with the DC reconstitution and clinical outcome after a myeloablative HLA-matched transplant. Fifty-three patients were included in this study, 37 undergoing a G-CSF-mobilized peripheral blood stem cell (PBSC) transplant from related donors, and 16 undergoing a marrow transplant from unrelated donors. The number of CD34+ cells, lin-HLA-DR+CD11c- mDC, lin-HLA-DR+CD123+ pDC, CD14+ monocytes, and CD3+CD4+, CD3+CD8+, CD56+, CD19+ lymphocytes was compared in the graft, as well as in the peripheral blood after transplant, in patients receiv-
ing more than, versus less than or equal to the median number of CD34+ cells in PBSC (5.78×10^6/kg), or in marrow (2.8×10^6/kg). A higher CD34+ cell dose was associated with larger numbers of mDC in PBSC (p=0.01), and pDC in marrow grafts (p=0.004). However, neither mDC nor pDC recovery after transplant correlated with the number of CD34+ cells infused. Finally, higher doses of CD34+ cells appeared to negatively affect (p=0.02) the overall survival in PBSC transplantation, and were associated with a trend of higher acute GVHD in PBSC, and lower acute GVHD in marrow transplant. The CD34+ cell dose correlates with the dose of different DC subsets in PBSC and marrow grafts, but it does not affect DC reconstitution after transplantation. Higher doses of CD34+ cells in PBSC, but not in marrow, seem to adversely affect survival after transplant.

Data on the role of HLA DP molecules in transplant outcome are contradictory. It is difficult to differentiate the role of DP molecules from that of other HLA molecules as this requires the availability of genoidentical donor/recipient (D/R) pairs different at the DP locus for a rare event of crossing-over. Alternatively, an optimal model for this type of evaluation is unrelated D/R pairs identical at the DP locus but sharing two extended haplotypes. In fact, in HLA-matched unrelated individuals, the entire structure of an HLA extended haplotype is generally identical except for rare variations at the centromeric and telomeric extremities. To evaluate the role of HLA-DPB1 molecules in GVHD and rejection, we studied 60 thalassemia patients transplanted from unrelated donor stem cells. At present, allogeneic SCT is the only definitive treatment available to patients with thalassemia major. These considerations make it important to avoid procedures that may compromise the fetal immune system and therefore susceptible to induction of immunologic tolerance to foreign antigens. So far, the best results have been obtained in USCTs carried out on fetuses with immunodeficiency disorders in which a selective advantage for donor stem cells is present. The results obtained in thalassemia have been much less satisfactory. A controversial issue, critical to the employment of USCT in thalassemia, concerns the feasibility of inducing fetal tolerance to transplanted donor stem cells. At present, allogeneic SCT is the only definitive treatment available to patients with thalassemia major. These considerations make it important to avoid procedures that may compromise the advantages offered by allogeneic SCT performed after birth. We report three unsuccessful cases of in-utero transplantation in β-thalassemia patients. All three patients were treated with allogeneic SCT after birth. In Case n°1, the fetus received 5×10^6 CD34 cells from...
the HLA-haploidentical father at the 30th week of gestation. PCR analyses of chimerism and synthesis of β chains performed at birth showed no signs of engraftment. At the age of 6 years, the patient was successfully treated with allogeneic SCT from an unrelated HLA identical donor. In case no 2, the fetus received a total of 3×10^6 CD34 positive cells (at the 12th, 14th and 16th week of gestation) from the HLA haploidentical father. Also in this case there were no signs of engraftment after birth. At the age of three years, the patient was successfully treated with unrelated HLA identical SCT. In case no 3, the fetus received a total of 15.6×10^6 CD34 positive cells from an HLA geno-identical sibling at the 14th and 16th week of gestation. At birth, a low level of chimerism was detected but the effect was transient and the patient remained transfusion dependent. After 3 years the patient was transplanted from the same HLA-genoidentical donor. The transplant was rejected followed by an autologous reconstitution. These 3 different cases of USCT in thalassaeemia patients seem to suggest that the use of the same donor in USCT and post-natal SCT may compromise the outcome of alloge nic SCT performed after birth.

PU218
ACUTE MEDIASTINITIS IN A PATIENT WITH ACUTE MYELOID LEUKEMIA UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION
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Acute mediastinitis is an uncommon, but life-threatening, complication involving the structure of mediastinum and is generally due to infection. Most frequently this event occurs as a post-operative infection following median sternotomy, while it is rare in hematologic malignancies. Whatever the pathogenesis, in the majority of cases the causes are secondary to spread of infection from other sites, or to direct inoculation by a trauma. Common clinical findings are fever, chest pain, and neck swelling. Early in the course of mediastinitis signs and symptoms may be subtle, but, as the condition progresses, increasing chest pain, respiratory distress, and dysphagia appear. In the setting of malignancies, a particular finding is the so-called chemical mediastinitis, due to leakage of cytotoxic agents or parenteral nutrition fluid from a migrated or broken central venous catheter. We report a case of acute mediastinitis associated with superior vena cava thrombosis in a patient undergoing allogeneic stem cell transplantation. A 43-year-old man, affected by diabetes mellitus, obesity and previous cardiac stroke, was diagnosed as having acute myeloid leukemia in March 2000. Complete remission (CR) was achieved after treatment with the FLAG regimen (fludarabine, cytarabine, G-CSF). Consolidation with an additional FLAG course was given and, after successful stem cell collection, the patient underwent autologous stem cell transplantation (SCT). The conditioning regimen consisted of busulphan plus melphalan. Neither documented infections nor other severe toxicities occurred after induction/consolidation chemotherapy or autologous transplantation. In April 2002 hematologic relapse occurred; a second CR was achieved with fludarabine and cytarabine as continuous infusion + daunoxome and, after consolidation with an identical course, on January 2003 the patient was admitted to be given reduced intensity allogeneic SCT from an HLA identical sibling. The conditioning regimen included fludarabine, cyclosporinamide, idarubicin and etopoïde, while the prophylaxis of GvHD consisted of cyclosporine and methotrexate. All drugs and total parenteral nutrition were administered through a central venous catheter (CVC) positioned in the right jugular vein. On day +13, the patient presented fever, dysphonia, neck pain and edema, and dyspnea. Chest X-rays was normal; echo-Doppler showed a thrombosis of right jugular vein. The CVC was removed and systemic anticoagulation and antibiotics therapy were started. Either blood culture or CVC tip culture were negative. While full engraftment was documented by bone marrow examination and hematopoietic recovery, the patient’s clinical status did not improve, therefore on day +18 a chest CT scan was performed, suggesting a diagnosis of acute mediastinitis. The patient underwent thoracotomy with surgical drainage of a small amount of fluid material, which was negative at coltural examination, and debridement. A rapid improvement of clinical status was observed in the following days, with disappearance of fever and swelling, and resolution of the venous thrombosis. We conclude that acute mediastinitis should be considered in high risk patients, particularly when a CVC is positioned. Surgical intervention represents a curative option which must be considered even in critically ill patients.

PU219
CYCLOSPORINE NEUROTOXICITY DURING VORICONAZOLE TREATMENT AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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A 24 years-old female patient, with high-risk ALL received an allografting in CR1 from her HLA-identical
sister after conditioning regimen with TBI and Melphalan. Cyclosporine (CyA) in combination with Methotrexate, was used as graft-versus-host-disease (GVHD) prophylaxis. On day +22 she developed grade II hepatic aGVHD, and steroids at dose of 2 mg/kg po qd was started with progressive improvement of hepatic functionality. On day +53, at steroid dosage of 0.4 mg/kg po qd, sinusitis suggestive for fungal infection was diagnosed and empirical antifungal therapy with Voriconazole at 400 mg po qd was started. On day +54, for abnormal serum levels of creatinine (1.83 mg/L) and high blood levels of CyA (504 ng/mL), CyA was reduced from 175 mg to 150 mg po tid and Voriconazole to 200 mg po qd, with return of CyA blood levels in a therapeutic range. Nevertheless, on day +60 patient needed hospitalization for onset of frontal headache, numbness, double vision, palsy of the sixth and seventh right cranial nerves, distal tremor and unsteadiness without meningeal signs. In the following days she developed disperceptions at both hands (feeling objects bigger than in reality), for wich it has been postulated an epileptic origin. Patient underwent a lumbar puncture; the cytological exam was negative, a basal electroencephalogram (EEG) showed symmetrical alterations, and brain magnetic resonance (BMR) that showed diffuse signal alteration in the brainstem, third ventricle walls and subcortical white matter. Cyclosporine A was withheld and replaced with mycophenolic acid (MMF) at 15 mg/kg tid on day +65; at the same time voriconazole was discontinued. After 2 days, the patient developed a generalized convulsion and therapy with Phenobarbital was started; EEG showed symmetrical alteration of the organization, left temporal slow focal abnormalities in the absence of epileptic signs. Clinical neurologic signs improved in the following two weeks, and EEG and BMR performed one month later were normal. We assume that an interaction between CyA and voriconazole has occurred in this patient, resulting in abnormally high levels of CyA, and subsequent neurologic toxicity. Voriconazole is a new potent antifungal compound that is rapidly absorbed after oral administration; it could be used also as empirical antifungal therapy after HSCT in which drug interaction with CyA need to be investigated.

PU220

**GRAFT-VERSUS-TUMOR EFFECT FOLLOWING REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN SOLID TUMORS**


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Introduction. Evidence is accumulating that a graft-versus-tumor effect might be induced in patients with solid tumors by means of allogeneic transplantation; early data indicate that cytotoxic T cells of donor origin are the mediators of this effect. Methods and Patients. Since 1999, forty-one patients with solid tumors have been transplanted in our institution (18 renal cell cancer, 9 breast cancer, 7 ovarian cancer, 4 prostate cancer, 1 colon cancer, 1 Ewing sarcoma and 1 yolk sac tumor). The median age was 51 years (range 18-66). The conditioning regimen included thiopeta (5 or 10 mg/kg according to diagnosis), fludarabine (60 mg/m²) and cyclophosphamide (60 mg/kg). All the patients received allogeneic peripheral blood cells from an HLA-identical sibling. A median of 5.3×10⁶ CD34+ cells/kg were infused (range 0.8-11.8); no positive selection, T-cell depletion or cryopreservation procedures were performed. GVHD prophylaxis consisted of cyclosporine A starting from day -6 (target range between 150 and 300 ng/mL) and short-course methotrexate (10, 8, 8 mg/m² at day +1, +3, +6, respectively). G-CSF was given from day +7. Thirty-eight patients were evaluable for engraftment and chimerism. The median time to reach an absolute neutrophil count of 1.0×10⁹/L and a platelet count of 20×10⁹/L was 13 and 14 days, respectively (range 10-15 and 11-19). Thirty-two patients achieved complete chimerism at day +60 by conventional cytogenetic analysis or by PCR-based analysis of VNTR polymorphisms in sex-matched pairs, but they all became full-donor after cyclosporine A withdrawal. The median day of cyclosporine A withdrawal was +110. Thirteen patients developed aGVHD/grade 2, usually soon after cyclosporine withdrawal. At a median follow-up of 240 days (range 8-1377), 14 patients are alive and 27 died, 23 for disease progression, and 4 for non-progression-mortality. We observed tumor response in 12 patients (7 renal cell cancer, 2 breast, 2 ovarian and 1 prostate cancer); the responses were usually concurrent with the development of GVHD. Duration of responses was 2-13+ months. Four patients received up to three infusions of donor lymphocytes: in one patient we observed a PR after DLI. Conclusions. Our data confirm the feasibility of a reduced-intensity allogeneic transplant in solid tumors with a very low non-progression-mortality (10%). The evidence of graft-versus-tumor effect in 30% of the evaluable patients and the promising overall survival warrant further investigations.

PU221

**GVHD AND CYCLOSPORINE A LEVELS IN BLOOD IN DIFFERENT MOMENTS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION: AN OPENED QUESTION?**

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Background and Objectives: GVHD is a major complication after allogeneic bone marrow transplantation (BMT), especially that from unrelated donor (MUD). Cyclosporine A (CyA) has a great importance in the prevention of chronic and acute GVHD. In this study we evaluated the correlation among blood CyA levels during the first 5 months post-BMT, prophylaxis duration and GVHD. Design and Methods: We retrospectively analyzed 57 patients underwent MUD-BMT during the period 06/1997 - 12/2002. Patients characteristics are shown in Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>62</td>
</tr>
<tr>
<td>Female</td>
<td>37</td>
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<td>Diagnosis</td>
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<td>ALL</td>
<td>24</td>
</tr>
<tr>
<td>CML</td>
<td>20</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>ST</td>
<td>2</td>
</tr>
<tr>
<td>MM</td>
<td>3</td>
</tr>
<tr>
<td>Disease status of BMT</td>
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</tr>
<tr>
<td>1CR or CML-C</td>
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</tr>
<tr>
<td>II or further CR or CML-A/B</td>
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<tr>
<td>Source of stem cells</td>
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</tr>
<tr>
<td>BM</td>
<td>75</td>
</tr>
<tr>
<td>PB</td>
<td>25</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
</tr>
<tr>
<td>TBI/CY</td>
<td>91</td>
</tr>
<tr>
<td>BU/CY</td>
<td>7</td>
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<tr>
<td>CML radiation</td>
<td>30</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>67</td>
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<tr>
<td>Grade-II/IV</td>
<td>25</td>
</tr>
<tr>
<td>N° of patients survived at least 3 months</td>
<td>75</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>75</td>
</tr>
<tr>
<td>Extensive</td>
<td>43</td>
</tr>
</tbody>
</table>

GVHD prophylaxis included, for all patients, methotrexate (MTX) (15 mg/m² on day +1, 10 mg/m² on day +3, +6 and +11) and CyA (3 mg/kg/d i.v. from day -1, then orally as soon as possible); CyA was tapered from fifth month or in a different moment according to the extension of chronic GVHD. In some cases, for particular clinical needs, one or two administrations of MTX were suspended. CyA levels in full blood were monitored using a monoclonal antibodies assay each day until +30, then 2 times a week, and they were expressed as monthly mean plus standard deviation. Results: We found significantly lower levels of CyA in patients with grade III-IV acute GVHD than in patients with grade I-II acute GVHD during first month after BMT (333±35 vs. 373±44, p=0.009). In the same period we found significantly higher levels of CyA in patients with extensive chronic GVHD than in patients with limited chronic GVHD (382±48 vs. 342±42, p=0.009). During 4th and 5th month CyA levels were significantly lower in patients with extensive chronic GVHD than in patients with limited chronic GVHD 4th month: 125±57 vs. 202±67, p=0.0006; 5th month: 109±48 vs. 176±90, p=0.04). Univariate analysis (Table 2) identify CyA levels in 1st month as possible risk factor for the severity of acute GVHD and for the incidence and extension of chronic GVHD; CyA levels in 4th and 5th month could be risk factor for the extension of chronic GVHD. Other results of univariate analysis are shown in Table 2.

The suspension of CyA administration before or during 6th month post-BMT is significatively correlated with the extension of chronic GVHD. Conclusions and discussion: previous works evidenced the central role of dosage and duration of the administration of CyA in GVHD prophylaxis. In our pool of cases, omogeneous regarding transplantation setting (MUD), conditioning regimen and GVHD prophylaxis, we evidenced that: 1) During the 1st month too low levels of CyA could increase the grade of acute GVHD and too high levels of CyA could promote the development of extensive chronic GVHD; 2) During the 4th and 5th month too low levels of CyA could facilitate the extension of chronic GVHD. It is possible that repetitive fluctuations between high levels of CyA at first and low levels therefore could facilitate these immunological complications. CyA is known to affect immunological tolerance in many ways. Its effects could be dose-dependant, so that during 1st month too high levels could interfere with the development of peripheral and central immunological tolerance. The main goal of further
investigations should be the definition of ideal modulation of CyA levels in different periods after BMT related to the development of immunological competence.

PU222
ALLOGENEIC STEM CELL TRANSPLANTATION AS SALVAGE THERAPY FOR PATIENTS WITH LYMPHOMA

Despite the therapeutic progress reached in the management of lymphoma, a minority of patients with Hodgkin lymphoma (HL) and the majority of patients with non-Hodgkin's lymphoma (NHL) do not reach lymphoma eradication with conventional therapies and die for progressive disease. The use of allogeneic stem cell transplantation (SCT) may represent an important salvage treatment, particularly for younger patients. We here report the results of a retrospective analysis performed on 23 patients with primary refractory or relapsed lymphoma who underwent allogeneic SCT between May 1994 and January 2003. Table 1 summarizes the patients' main clinical and transplant characteristics. This case record included 4 patients with HL (2 with chronic lymphocytic leukemia (CLL), 5 with follicular lymphoma (FOL), 6 with diffuse large B-cell lymphoma (B-DLCL), 6 with peripheral T-cell lymphoma unspecified (PTCL-U)). The median number of treatments received previous SCT was 3 (range 1-5). The clinical status before SCT was: CR 3 patients, PR 8, progressive (PRO) or non-responsive (NR) disease 12. Seventeen patients underwent SCT with a sibling HLA full-matched donor while 6 with a full-matched unrelated donor (MUD). Seventeen patients received a conventional conditioning regimen based on the administration of chemotherapy or chemotherapy plus total body irradiation (TBI), while 6 patients received a reduced intensity conditioning (RIC) regimen. The prophylaxis of acute GVHD was conducted using cyclosporin-A (CSA) and methotrexate (MTX) or CSA and micofenolate (in some patients who underwent RIC). The treatment-related mortality rate (TRM) was 39% (9 patients), 47% (8/17) in the conventional group and 17% (1/6) in the RIC group. One patient died 4 months after SCT because of lymphoma progression. After SCT 14/23 (61%) patients achieved a CR, 1 a PR and 2 were NR or PRO; in 6 patients the status after SCT could not be evaluated because of early mortality. Grade III-IV acute GVHD was registered in 2 patients and extensive chronic GVHD occurred in 4 out 16 valuable patients (25%). After a median follow-up after SCT of 7.5 months (range 1-108), 14 out 15 responders remain relapse/progression free and the OS rate is 56%. This preliminary analysis indicates that allogeneic SCT (either with conventional or RIC) is a feasible and effective salvage therapy for patients with lymphoma. All patients considered in this analysis failed prior conventional therapies (including autologous SCT in 8) and would have been eligible only for palliative medications. In these cohort of patients, on the contrary, allogeneic SCT allowed the achievement of a prolonged remission in nearly 50% of cases. These results seem to encourage the use of allogeneic SCT in patients with no other curative conventional therapeutic options.

PU223
MINIMAL RESIDUAL DISEASE EVALUATION IN CHRONIC MYELOID LEUKEMIA PATIENTS SUBMITTED TO ALLOGENEIC BONE MARROW TRANSPLANTATION BY REAL TIME PCR

The BCR-ABL chimeric protein, determined by the t(9;22) translocation, is the molecular hallmark of CML. Therefore, it is a very useful marker to monitor MRD in CML patients submitted to various treatment procedures, especially allo-BMT. In past years, the molecular method most frequently used to evaluate MRD was qualitative reverse transcription (RT-PCR). More recently, it has been demonstrated that real time RT PCR is one of the most effective methods to quantity the amount of BCR-ABL residual leukemic cells. Therefore we have used this approach to evaluate 43 CML patients who received an allo-BMT at our Institution. In order to monitor product accumulation during PCR reaction, relative quantification of BCR-ABL transcripts was performed by real-time PCR using SybrGreen I as double-stranded DNA binding fluorescent dye. Both forms of p210 BCR-ABL mRNA transcripts (b2a2 and b3a2) were detectable with the same set of oligonucleotides by analysing dissociation curves. A serial dilution of total RNA from K562 cells was used to construct a standard curve for real-time quantification. The sensitivity threshold for BCR-ABL mRNA quantification was fixed at 10^{-4} dilution standard mRNA, corresponding to 6pg of RNA. BCR-ABL expression levels were normalized to ABL mRNA expression and calibrated on K562. Forty patients were transplanted in first chronic phase (CP), three in an accelerated phase (AP). Thirty-five patients received a marrow from a sibling while the remaining from an unrelated donor. In nineteen patients the donor was sex-mismatched. Thirty patients (69,8%) transplanted in CP presented constantly negative qualitative and quantitative RT PCR
tests and achieved a persistent molecular and hematologic remission; fifteen transplanted from a sex-mismatched donor were full donor chimeras. The remaining thirteen patients (30.2%) presented at least one qualitative RT PCR test either within the first six months posttransplant or after one year. On real time PCR ten of them showed a reduction of BCR-ABL/ABL ratio to undetectable levels. This event was also observed in the two patients with persistently long-term positive qualitative RT PCR assays. None of these patients experienced clinical relapse. In contrast, the three patients transplanted in AP had a progressive and significant increase in the amount of the BCR-ABL/ABL ratio. A clinical relapse occurred in all of them. In conclusion our data show that real time PCR provides important information in CML patients submitted to allo-BMT with a constantly positive BCR-ABL qualitative PCR, allowing the identification of those having a high relapse risk.

PU224
ALLOGENIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOLLOWING A NON-MYELOABLATIVE (NMST) CONDITIONING WITH FLUDARABINE AND LOW DOSE TBI IN A PATIENT WITH MYELOFIBROSIS WITH MYELOID METAPLASIA
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Myelofibrosis with myeloid metaplasia (MMF) is a clonal hematopoietic disorder characterized by bone marrow fibrosis, a leukoerythroblastic blood picture, splenomegaly, and extramedullary hematopoiesis. Most patients are being diagnosed at 50 to 69 years of age. Median survival ranges between 3 to 5 years, and conventional therapies are often ineffective and only palliative. Allogenic hematopoietic stem cell transplantation in patients older than 45 years old resulted in a 14% 5-year overall survival in a recent update of a study conducted on 55 patients, with 1-year TRM of 27%. In a recent study 4 patients have been transplanted with a reduced intensity conditioning and all patients engrafted. We describe a case of a 53 years old male with MMF who received a NMST from an HLA-identical sibling. The patient before transplant had anemia (10 g/dL), splenomegaly (25 cm) extensive marrow fibrosis, and increasing LDH (2230 U/L) over the last months. The basic conditioning regimen included fludarabine 30 mg/m² on day -4, -3 and -2, and low-dose TBI (200 Gy) and postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSA). The patient received an unmodified peripheral stem cell graft from an HLA identical sibling G-CSF mobilized. Matching was by serological analysis for HLA-A, B and C, and by molecular matching for HLA-DRB1. Patient and donor were ABO matched. The patient received 7.0×10⁶ unmanipulated CD34 cells/kg. Donor chimerism was assessed among blood T cells, granulocytes and unfractonated bone marrow by polymerase-chain-reaction-based analyses of variable number of tandem repeat. The patient experienced no aGVHD grade II to IV, no mucositis and no alopecia. Chimerism analysis at day +28 revealed donor CD3 66%, donor granulocytes 94% and on unfractonated bone marrow 86%. The patient required 5 units of packed red blood cells and 4 units of platelets. All blood products were irradiated. The patient is now 141 days post transplant, in good performance status, with reduced splenomegaly (from 25 cm pre-transplant to 17 cm post-transplant), Hb 9.0 g/dL, WCC 3,000/µL, PLT 110,000/µL, with donor chimerism assessed on CD3 and granulocyte compartment and bone marrow showing 59%, 90% and 88% respectively. Chronic GVHD assessment revealed subclinical cGVHD of the skin. Although a longer follow up is needed and a wider number of patients is necessary in controlled trials to draw any conclusion, the patient transplanted with a non-myeloablative conditioning experienced a mild toxicity, LDH and splenomegaly are decreased substantially and chimerism analysis, despite marrow fibrosis, revealed engraftment.

PU225
ACUTE GRAFT VERSUS HOST DISEASE IN A CML PATIENT WHO RECEIVED A SYNGENIC PERIPHERAL BLOOD STEM CELL TRANSPLANT
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A 50 years old female with CML chronic phase was initiated on a tril with STI-571 at 400 mg po every day. The patient was assessed by RT-PCR every two months. Six months later she was Ph negative and in molecular remission with RT-PCR negative for BCR/ABL transcript. The patient received than a syngenic myeloablative peripheral blood stem cell transplant. The patient was conditioned with BU/Cy and no aGVHD prophylaxis. The patient received 4.0×10⁶ CD34/kg from her syngenic donor G-CSF mobilized. The patient experienced mucositis grade III WHO wich required i.v. analgesia, no aGVHD and no VOD were detected. Engraftment considered as the first of two consecutive days with ANC > 500/µL and PLT > 20,000/µL were respectively at day +11 and day +10. Only complain was mild fluctuating nausea which was considered to be conditioning-related. The patient was discharged with relief of gastric symptoms on day +18. She experienced no bacterial, no fungal and no viral infections. CMV antigenemia was monitored and resulted always nega-
tive. The patient is now 95 day from the transplant and is on follow up in our out-patient Unit. The patient was monitored by RT-PCR at day +21, +60, +90 and all controls were negative. On day + 50 she complained loss of appetite and mild nausea, in absence of skin, liver or lower gastrointestinal tract aGVHD. Because the symptoms did not improve with metoclopramide and or benzodiazepin, the patient underwent endoscopy with multiple stomach biopsies. The endoscopy revealed hyperemic mucosa, without ulcers. The histology revealed apoptotic bodied, focal epithelial necrosis localized to the base of gastric pit. No CMV was noted in the samples analyzed. The histology picture was consistent with mild stomach aGVHD. The patient was started on steroid treatment at 2 mg/kg with prompt resolution of symptoms. The patient is now undergoing assessment for cGVHD. Acute GVHD is infrequent after syngenic stem cell transplantation, yet a careful search of aGVHD and/or cGVHD has to be done in the presence of symptoms that might be confused even with drug toxicity. The patient is BCR/ABL negative by RT-PCR.

CML patients undergoing syngenic transplant have more probability of relapse in respect to allogenic transplants from an HLA matched sibling donor. Although this is true a certain graft- versus leukemia effect is still present. Because acute and chronic GVHD are both associated with a lower relapse rate in the setting fo allogenic transplants, we might speculate that a mild aGVHD might improve the chances of a longer RFS in this patient.

PU226
SUCCESSFULL TREATMENT OF PURE RED CELL APLASIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH RITUXIMAB
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Pure red cell aplasia is a well known complication after major ABO incompatible allogeneic stem cell transplantation and seems related to the inhibition of growth of donor erythroid progenitors caused by the persistence of natural anti-A or anti-B recipient antibody. Treatment relies on steroids with or without erythropoietin, extended immunosuppression, plasma exchange or donor stem cell boosting. Recovery of erythropoiesis is difficult and often incomplete to achieve and treatment itself may induce further complications. Rituximab has been shown to obtain consistent result in refractory autoimmune cytopenias and a recent case report has been published showing the successful treatment of PRCA after SCT with a single dose of rituximab in a child. On the basis of these results we report a 53-year-old female with PRCA after ABO incompatible allogeneic SCT for secondary acute leukemia. The patient was O Rh+ and her brother was AB Rh+. After conditioning regimen with busulfan and cyclophosphamide she received a graft containing 6.3 CD34+ ×10^6/kg from her HLA-identical donor. GVHD prevention included cyclosporine A and methotrexate. The post-transplantation course was unremarkable except for persistent anemia and a extremely low reticulocyte count. Bone marrow cellularity was normal unless for erythroid precursors which were 1-2% of overall cellularity. During follow-up the patient remained anemic with low reticulocytes 0.1-0.2 and requiring at least 1 unit of RBC/week. Erythropoietin at the dosage of 10.000 u/every other day was added by day +55 after transplantation, but was not effective in reducing the transfusion requirements. Considering the long-standing PRCA and the absence of response to steroids, cyclosporine and erythropoietin, a second stem cell donation with CD34+ cell selection was planned to overcome PRCA. In the mean time the temporary unavailability of the donor prompted us to administer rituximab at the single total dose of 400 mg. After one week no response was observed, reticulocyte count was 0.2% then a second and a third and final dose was administered. After the second dose of rituximab reticulocyte count promptly increased to 2.4% and hemoglobin levels raised from 6.6 g/dL to 11.4 g/dL after the third dose of rituximab. Bone marrow cellularity was normal and erythroid precursors rose up to 8% after the second dose of rituximab. Epo was stopped at day +170 after the third administration of Rituximab. The patients is on day +200 after transplantation with limited signs of cGVHD signs, with complete hematologic recovery and normal erythroid maturation. The outcome of this patient showed that the use of anti CD20 monoclonal antibody is a promising treatment modality for patients affect by PRCA after ABO incompatible allogeneic stem cell transplantation. This approach was effective and safe sparing unwanted side effect related to immunosuppression and steroid-dependence. Although a low dose of rituximab was used the complete recovery of erythroid lineage was promptly obtained and this was also cost-effective. Larger number of patients could permit to definitively validate this treatment as a first line choice of PRCA after allogeneic stem cell transplantation.

PU227
ALLOGENEIC BONE MARROW TRANSPLANTATION IN SECONDARY ANAPLASTIC T-CELL LYMPHOMA DERIVED FROM MYCOSIS FUNGOIDES: A CASE REPORT
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Mycosis fungoides (MF) is the most common type of cutaneous T cell lymphoma. MF is usually an indolent disorder, but approximately 20% of the patients with advanced stage MF undergo large cell transformation. Large cell transformation of MF bears a poor prognosis and usually heralds the terminal stage of the disease. For these patients conventional chemotherapy does not have an impact on survival whereas autologous bone marrow transplantation, that has been performed only in a few patients, is associated with a high relapse rate in spite of extensive ex vivo purging. Allogeneic stem cell transplantation is theoretically attractive for several reasons, such as the observed response of MF to a variety of treatments which are known to modulate immune response, and the possibility of a graft versus tumor effect. We describe a case of patient with anaplastic T cell cutaneous lymphoma secondary to MF, who underwent nonmyeloablative allogeneic stem cell transplantation. Case report: a 51 years old male patient was diagnosed with plaque stage MF in 1997. Initial treatment consisted of PUVA plus α interferon. In 1999 a progression to tumor phase mycosis fungoides was observed, and a biopsy specimen was consistent with secondary CD30+ ALK- anaplastic large cell lymphoma. Since then, the patient tried several lines of therapy (gemcitabine, CHOP, MOP/EBV/CAD, DHAP): the disease, although chemosensitive, was characterized by early relapses after each line of therapy. On November 2002, in a minimal disease state, the patient underwent bone marrow transplantation from his HLA identical sister. Due to major ABO incompatibility (R/D = 0/AB), 3 plasma-exchange procedures were performed before transplant. Conditioning regimen included Thiotepa 10mg/kg on day -6, cyclophosphamide 30mg/kg on days -4 and -3, and fludarabine 30mg/mq on days -4 and -3. GVHD prophylaxis was based on cyclosporine and methotrexate. The whole treatment was well tolerated, and engraftment took place on day +13. On day +16 (positive CMVAg) pre-emptive treatment with ganciclovir was started. Peripheral blood CD3+ mononuclear cells and bone marrow cells were >95% of the cells. More than 95% red blood cells on day +165 were of donor origin (AB group). Following conditioning the patient achieved a complete response. On day +26, however, some small new plaques were observed. Cyclosporin was tapered on day +45, and the plaques resolved. On day +90 new lesions were evident; thus, CyA was withdrawn on day +123 and donor lymphocytes were infused on days +168 and +200. A partial response has been observed and, at present, there is no evidence of GVHD. Although DLI is still an ongoing programme and a longer follow-up is necessary for this patient, these data confirm the existence of a graft versus tumor effect in secondary anaplastic cutaneous T-cell lymphoma.
3 patients CMV reactivation occurred before the onset of ECP and promptly responded to pre-emptive therapy. It has been suggested that the presence of expanded clonal T-cell populations is predictive of good response to ECP in patients with chronic GVHD, and we actually found detectable T-cell clones in 2 out 5 patients (not included in the present report) with cGVHD. In these 4 patients with acute GVHD, however, we failed to detect clonal TCR gene rearrangements. Although the mechanisms of action of ECP are still unknown, our preliminary results suggest that ECP is an effective and safe complement of treatment for resistant acute GVHD and might have a role in first line therapy of acute GVHD.

PU229
SEVERE DEFICIT OF CLONOGENIC FIBROBLAST PROGENITORS MAY BE INVOLVED IN THE PERSISTENT BONE DAMAGE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION
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In the last few years, it has become clear that bone damage represents a frequent and serious complication after allogeneic stem cell transplant (allo-SCT). Discussion on the pathophysiology of post transplant bone loss always rely on the main contributing factors: age, immunosuppressive treatments and hypogonadism. We investigated in vitro recovery of clonogenic fibroblast progenitors (colony forming units-fibroblast cells, CFU-F), which represent a pivotal step of bone remodeling, in 35 long-term survivors after allo-SCT and compared it to bone mass after allo-SCT detected at three skeletal sites by dual energy x-ray absorptiometry and osteoscinometry. For CFU-F assay, bone marrow mononuclear cells from 35 patients and from 20 normal donors were resuspended at a concentration of 2×10⁶/mL in McCoy’s 5A modified medium supplemented with 1×10⁻⁴ mol/L dexamethasone, which allows the recruitment of bone marrow mesenchymal cells to the osteoblastic lineage, and plated at 37°C, 5% CO₂ for 14 days. Osteoblastic differentiation of the colonies was defined by their ability to express alkaline phosphatase activity. Marrow stromal layer was also analyzed for stromal confluence after 4-5 weeks of culture. All transplanted patients showed at the time of testing complete engraftment at hemopoietic and at molecular level. The marrow compartment of stromal cells, measured as CFU-F cells, was decreased 2 to 3-fold in transplanted patients compared to normal donors (22.3±3×10⁶ MNC plated vs 55±4; p<0.0001). In transplanted patients, the number of CFU-F cells was not related to the number of myeloid progenitors infused: using as cut-off the median value (2.93±10⁹/kg myeloid progenitors), there was no difference in CFU-F frequency between the groups of patients who had received more or less myeloid progenitors (p =0.74).

After 5 weeks of long-term culture, marrow cells produced a confluent marrow stroma only in 20% of cases, compared to 80% in normal controls. Analyzing the effect on marrow CFU-F number of the time elapsed since transplantation, we found the marrow CFU-F compartment markedly depleted during the first 6 years after transplant. Between six and ten years, the mean marrow CFU-C cell number tended to increase (13.28±3.48 vs 33.30±5.36 before and after 67 months, respectively; p=0.004), although most patients showed a number of CFU-F permanently below that observed in normal controls. CFU-F growth correlated significantly with lumbar, femoral and phalangeal bone loss (p<0.01, <0.001 and = 0.003, respectively). The grade of acute GVHD did not affect the number of CFU-F after SCT (p=0.38; p =0.06 and p=0.43 for grade I, II and III-IV). Chronic GVHD was correlated with a lower number of CFU-F colonies in vitro (p=0.002). Finally, almost all transplanted patients who had osteonecrosis showed a number of CFU-F below that observed in transplanted patients without this complication (CFU-F: 24.5±3.5 vs 12.4±4.3 in allo-SCT patients without and with osteonecrosis, respectively; p=0.042). In conclusion, our study has documented a severe and permanent deficit of number and function of osteoblastic precursors in the stromal cell compartment, suggesting that inability to regenerate a normal osteogenic cell compartment may account at least in part for severe bone damage after allo-SCT.

PU230
REDUCED INTENSITY STEM CELL TRANSPLANTATION DOES NOT IMPROVE OUTCOME FOR OLDER PATIENTS WITH ACTIVE HEMATOLOGIC MALIGNANCY AT THE TIME OF TRANSPLANT
Division of Hematology, Federico II University of Naples, Italy

High transplant-related mortality (TRM) rate remains a major obstacle for elderly patients to successful myeloablative allogeneic stem cell transplantation (allo-SCT). Non-myeloablative and reduced intensity conditioning regimens (RI-CR) have decreased regimen-related mortality in this cohort of patients. However, relapse and graft-versus-host disease (GVHD) continue to be major causes of morbidity and mortality. We report the results of a RI-CR followed by peripheral blood (PB) or bone marrow (BM) allo-SCT from a HLA-identical sibling in 18 patients with a median age of 47 years (range 40–55). The underlying disease was CML
in chronic phase (n=3), CM L in blastic phase (n=1), AML in first complete remission (n=3), AM L resistant and in partial remission (n=6), MDS (n=2), myelofibrosis (n=1), NHL in leukemic phase (n=1) and ALL in partial remission (n=1). Nine of these patients (55%) had active disease at the time of transplant. The RI-CR consisted of thiotepa (THIO, 15 mg/kg) on day -6 and -5 or THIO (10 mg/kg) for patients with and without active disease, respectively, associated with cyclophosphamide (CY, 50 mg/kg) on days -3 and -2 (total dose 100 mg/kg). The source was BM (n=8) or G-CSF-mobilized progenitors (n=10), which were infused without manipulation. GVHD prophylaxis consisted of cyclosporin A and short course methotrexate. After transplantation, BM mononuclear cells were analyzed monthly for degree of donor-recipient chimerism using polymerase chain reaction (PCR) of minisatellite regions. Hematologic recovery was prompt in all cases. Neutrophils decreased to less than 0.5x10^9/L in all cases and recovered at a median of 20 days after transplantation (range, 14-27). Median time to reach a stable platelet count more than 20x10^9/L and more than 50x10^9/L was day +19 (range 0-36) and day +21 (range 14-51), respectively. Only 7 patients (38%) developed febrile neutropenia. Thus far 5 patients have reactivated cytomegalovirus infection. On day +100, 11/18 (60%) patients showed complete marrow donor chimerism. Grade III or IV acute GVHD occurred in 4/18 (22%) patients (3 after PB-SCT). Chronic GVHD was seen in 45% of patients, with a higher rate for PB (75%) compared with BM transplants (25%). For patients with CML in chronic phase, AM L in remission, myelofibrosis and MDS with stable disease, the actuarial TRM, relapse and survival were 0%, 22% and 100%, respectively. For patients with active disease at the time of transplant, the actuarial TRM, relapse and survival were 10%, 100% and 10%, respectively, although 5 of these 9 patients experienced a transient hematologic remission (mean 5.6 months, range 3-10). Three patients transplanted in active disease received donor lymphocyte infusion (DLI) for either persisting or relapsing disease; one of them experienced a prolonged complete remission, but then relapsed. Currently, 11 patients are alive at a median follow-up of 14 months (range 5-32) and 7 patients (40%) remain free of disease. In conclusion, elderly patients can be allografted (range 5-32) and 7 patients (40%) remain free of disease at the time of transplant, the actuarial TRM, relapse and survival were 10%, 100% and 100%, respectively. For patients with active disease, myelofibrosis and MDS with stable disease, the actuarial TRM, relapse and survival were 0%, 22% and 100%, respectively. For patients with CML in chronic phase, AML in blastic phase (n=1), AML resistant and in partial remission (n=6), MDS (n=2), myelofibrosis (n=1), NHL in leukemic phase (n=1) and ALL in partial remission (n=1). Nine of these patients (55%) had active disease at the time of transplant. The RI-CR consisted of thiotepa (THIO, 15 mg/kg) on day -6 and -5 or THIO (10 mg/kg) for patients with and without active disease, respectively, associated with cyclophosphamide (CY, 50 mg/kg) on days -3 and -2 (total dose 100 mg/kg). The source was BM (n=8) or G-CSF-mobilized progenitors (n=10), which were infused without manipulation. GVHD prophylaxis consisted of cyclosporin A and short course methotrexate. After transplantation, BM mononuclear cells were analyzed monthly for degree of donor-recipient chimerism using polymerase chain reaction (PCR) of minisatellite regions. Hematologic recovery was prompt in all cases. 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Leptin was measured in the serum by calculating in all subjects as weight divided by height. Leptin was prospectively evaluated with leptin determination in 60 controls, matched for age and body mass index (BMI) and F/M ratio. Compliance was excellent to all treatments administered. In conclusion, HRT, calcium and vitamin D supplements were not able to prevent bone loss in long-term survivors after allo-SCT. Either oral or intravenous bisphosphonates were effective in improving BMD at lumbar spine, and prevented bone loss at femoral neck. Therefore, bisphosphonates are necessary for prevention and treatment of osteoporosis in patients after allo-SCT.

PU232
SERUM LEPTIN IS ELEVATED AFTER HEMATOPOIETIC STEM CELL TRANSPLANT, ESPECIALLY WHEN CHRONIC GRAFT VERSUS HOST DISEASE IS PRESENT
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Leptin, an adipocyte-derived hormone of the long-chain helical cytokine family, is a signaling peptide first demonstrated to play a central role in the neuronal control of body fat. Several studies showed serum leptin levels increased after hearth, liver or kidney transplantation in relation with corticosteroid treatment, insulin levels and kidney function. Recently, the Th1 activating effect of leptin through the interaction with the long isoform of its receptor has been documented activating cytokine production and treatment of osteoporosis in patients after allo-SCT.

PU233
THE IMPACT OF RELAPSE ON SURVIVAL AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Relapse following an allogeneic HSCT remains a significant problem in patients with leukemia. We have studied the impact of leukemia relapse in 367 patients allografted between 1993 and 2002, with unmanipulated marrow or peripheral blood cells, in remission (1st or 2nd) at the time of HSCT. The diagnosis was AML (n=95), ALL (n=58) or CML (n=214). All patients were alive on day +100 to be at risk of relapse. Relapse was scored in 118 patients: their actuarial survival at 10 years is 47% and 71% for 249 patients who did not relapse (p<0.0001). There were however significant dif-
ferences between different leukemia: for ALL the actuarial 10 year survival for relapsed patients (n=22) is 10%, vs 70% for non relapsed patients (n=36) (p<0.0001). In AML actuarial survival is 17% for relapsed patients (n=26) and 90% for non relapsed patients (n=69) (p<0.0001). For patients with CML actuarial survival is identical: 67% for patients who relapsed (n=70) and 67% for relapsed patients (n=144).

This study suggests that therapeutic interventions which include discontinuation of immunosuppressive therapy, donor lymphocyte infusions (DLI) and a second transplant are effective in patients with CML, and have a reduced efficacy in patients with acute leukemia. The consequence is that at present it makes no difference if a patient with CML relapses after transplant, whereas for acute leukemias relapse seems to be a definitive event. Early diagnosis of relapse with molecular techniques may prove helpful in acute leukemia, since in this case DLI have been shown to be effective (Bone Marrow Transplant 2002; 30: 579-85).

INFECTIONS, QUALITY OF LIFE, SUPPORT THERAPY

Infections

PU234

HAFNIA ALVEI SEPTICEMIA IN HCV POSITIVE PATIENT WITH DIFFUSE LARGE B-CELL LYMPHOMA OCCURRING AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Hafnia alvei is a motile Gram-negative bacterium that belongs to the Enterobacteriaceae family; sometimes it may be found as part of the gastrointestinal flora but it is rarely isolated from human specimens. We report on a case of Hafnia alvei septicemia and abdominal abscess in an HCV positive patient (pt) with a diffuse large B-cell lymphoma occurring after an autologous stem cell transplant. The infection was successfully treated by imipenem without surgery or other invasive treatments (such as CT-guided percutaneous drainage). A 60-year-old woman complaining of left-side hypochondrial pain, fever, fatigue, loss of weight, and profuse sweating, was referred to our Institution in May 2000. Physical examination revealed only a severe splenomegaly. Blood count was normal and laboratory analyses showed only an increase of LDH and alkaline phosphatase; serology test for HCV was positive (1b genotype). In August 2000 she underwent splenectomy and a liver biopsy. Histological examination revealed a diffuse large B-cell lymphoma, involving the liver and spleen. The pt was treated with one cycle of chemotherapy according to the F-MACHOP regimen which was complicated by severe neutropenia and pneumonia. After this therapy she remained in observation without other treatments. In April 2001 for the progression of the lymphoma she received four courses of anti-CD20 antibody and three cycles of CHOP chemotherapy with a complete remission, followed by hematopoietic stem cell harvest (3x10^6/kg CD34+ cells), after priming with G-CSF and cyclophosphamide, with good clinical and hematologic tolerance. In July 2002 she underwent autologous PBSCT after BAVC as a conditioning regimen. Granulocyte colony-stimulating factor (5 µg/kg) was administered from day +4 to stimulate hematopoietic recovery. Antimicrobial prophylaxis consisted of trimethoprim-sulfamethoxazole and itraconazole. During the neutropenic period, on the fifth day after the reinfusion, she became febrile. Empirical antibiotic treatment was started with piperacillin-tazobactam.
150 mg/kg/day. Three days later the blood cultures became positive for Hafnia alvei that was sensitive to aminoglycosides, ciprofloxacin, imipenem, piperacillin and trimethoprim-sulfamethoxazole and resistant to amoxicillin and some cephalosporins. On day +10 despite the hematologic recovery, the pt remained febrile with a left hypochondrial pain. The physical examination showed increased tenderness in the upper left abdominal area without signs of peritoneal irritation. Abdominal computed tomography (CT) revealed the presence of abscess (6x5 cm) in the splenic recess. According to the sensitivity profile of Hafnia alvei the antibiotic therapy with piperacillin-tazobactam was replaced by imipenem (3 g/day) for a further 15 days with a progressive improvement of the clinical conditions and resolution of fever and abdominal pain. The pt was discharged on day +23 without symptoms. Later oral treatment with levofloxacin was given for 1 month. At the last follow-up in April 2003 she was still in complete remission and a total body CT scan was negative. Until now this is the first reported case of Hafnia alvei septicemia with an abdominal abscess in adult oncohematologic patients submitted to a bone marrow transplant. It also confirms the increase of unusual bacterial infections in this set of patients.

PU235
INTRACRANIAL CRYPTOCOCCOMA
Bertoncelli MC, Airoldi A, Bobbio F, Paccagnino L, Panagini D, Zigorri P, Campanini M
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Fungal infections are a frequent cause of morbidity and mortality in hematologic patients above all due to the increased application of immunosuppressant therapies in patients already immunocompromised by their disease. The diagnosis, which should be as early as possible, often presents a serious challenge because of the difficulty of achieving certainty. We report a case of a 48 year-old woman with non-Hodgkin’s follicular stage IV-A lymphoma, treated at onset in 1996 with FDN and retreated on relapse (April 2000) with HDCT + Rituximab + stem-cell autotransplant. In May 2000 headache, vomiting, fever and right arm clonospasm occurred; CSF cytology showed no lymphoma cells but only T-lymphocytes. India ink stain from CSF was negative. The blood tests for cryptococcus, aspergillus, CMV, EBV, herpes, toxoplasma screening were negative. Brain CT revealed left-frontal and right parietal-occipital hypodense lesions with perilesional edema; MR confirmed these data with hyperintensity on T2 weighted image enhanced with gadolinium, suggesting an infective origin of this finding. Treatment with liposomal amphotericin B and dexamethasone was start-
a bone marrow transplant (36 autologous and 13 allogeneic) and the other phases were for induction, consolidation, rescue, high-dose chemotherapy. The 2 groups were similar for diagnosis, disease-phase, chemotherapy or transplant, prophylaxis with levofloxacin (56 and 56 for each group), presence of CVC (43 and 37 for group 1 and 2 respectively). Each group consisted of 58 phases. The mean number of days with PMNs < 0.1×10⁹/L was 10.4 days (range 1-39) for the group 1 and 9 days (range 1-32) for the group 2. In each group there were 34 phases with at least one febrile episode but the number of total febrile episodes was 43 for the group1 and 37 for the second group. The mean days with fever was 8.4 for the group 1 and 5.6 for the group 2. The diagnoses of fever were respectively for group 1 and 2: FUO 22 vs 12, clinically documented 7 vs 3, microbiologically documented with bacteremia 7 vs 17, microbiologically documented without bacteremia 4 vs 1, neoplastic fever 1 vs 2 and bacteremia 7 vs 17. The isolated strains were without bacteremia 4 vs 1, neoplastic fever 1 vs 2 and bacteremia 7 vs 17, microbiologically documented with bacteremia 7 vs 17, microbiologically documented without bacteremia 4 vs 1, neoplastic fever 1 vs 2 and antibiotic-related fever 2 vs 2. The isolated strains were respectively for each group: gram positive bacilli 6 versus 10 (they were all methycilline-resistant coagulase-negative bacilli), gram negative bacilli 0 vs 7 (2 were Escherichia coli and 5 were Pseudomonas aeruginosa), fungi 5 vs 0. Considering the phases of each group the days of antibiotic therapy were respectively 9.8 (range 4-30) and 10.3 (range 4-36), in the first group 13 phases needed a second or third antibiotic line versus 8 in the second group. Antifungal therapy was administered in 12 phases of the group1 and in 5 phases of the group 2. All the febrile episodes except 6 resolved: of the 6 deaths 4 were in the first group and 2 in the second one. The 2 deaths in the group 2 were caused by gram negative bacilli while the 4 in the group 1 were caused by gram negative bacilli (1) and by fungi (3). Our work shows that the antibiotic treatment with Tazobactam/Piperacillin and Cefepime plus Amikacin of the febrile neutropenic patient is similar in terms of days of fever, need for further antibiotic lines, need for antifungal therapy and resolution of fever even if the small differences are not statistically significative because of the small number of patients.

PU237
HEMATOLOGIC PROFILE IN SUSPECTED SARS. CASE REPORT
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We describe a case of suspected ASRS (Acute Severe Respiratory Syndrome) observed in a child sent to Emergency Ward of Children’s Hospital IRCCS Burlo Garofolo and discharged after two weeks with diagnosis of probable atypical pneumonia. The subject was a female of eight years old, coming from Southeast Asia (Chine), hospitalized with fever (>38°C), headache and cough, immediately transferred in Infective Department. Visual count data by XE-2100 Dasit hemocytometer at the beginning indicated lymphopenia in neutrophilia. Patient reported these principal hematologic parameters: WBC 8.27 (10⁹/ul), RBC 4.74 (10¹²/ul), HGB 13.1 (g/dL), PLT 164 (10⁹/ul). Information from the differential count scattergram was: NEUT 6.60 (10³/uL-7.6%), LYMPH 0.94 (10³/uL-11.4%), MONO 0.63 (10³/uL-7.6%), EO 0.09 (10³/uL-11.1%), BASO 0.01 (10³/uL-0.01%). No alarm messages by display, no histogram and scattergram abnormalities and for dealing with instrument flags. We suggested another control of the same data hard copy. Computer scattergram analysis detected atypical lymphocytes described as OTHER alarm 0.2%. This signal advised the presence of abnormal lymphoid cells at this location demonstrating the excellent sensitivity of the DIFF channel in detecting abnormal lymphoid cells, confirmed by peripheral smear appearance, with traceability to the morphologic characteristics of the May-Giemsa stained blood film particularly for rarely occurring cells (large granular lymphocytes). The investigations for Coronavirus infection is in progress and tests found only Parainfluenza virus type 3 (PIV3). The signal OTHER at 0.2% is, we believe, an alert common in viruses and in normal samples too, but our goal is to verify this alarm by data hard copy in other suspected SARS admitted in hospitals and hematology services provided with hemocytometer DASIT XE-2100.

PU238
MENINGOENCEPHALITIS INDUCED BY LISTERIA MONOCYTOGENES IN PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS
Ronconi F, Pocali B,* Palmieri S,* D’Amico M R,* Annunziata M,* Volpe E, Ferrara F*
Division of Hematology, AORN “S. G. Moscati”, Avellino; *Division of Hematology and Stem Cell Transplantation Unit, AORN “A. Cardarelli”, Napoli

Opportunistic infections remain a major cause of morbidity and mortality in patients with lymphoproliferative disorders (LD). Impaired cellular or humoral immunity deriving from either malignancy itself or chemotherapy and/or steroids represents the main predisposing risk factors. We describe two patients suffering from multiple myeloma (MM) and chronic lymphocytic leukemia (CLL), respectively, who during the treatment of their disease developed severe meningoencephalitis by Listeria Monocytogenes. Case 1, DM, a 52-years-old male, was diagnosed on December 2002 as having MM of IgG-kappa type in stage III A according to Durie and Salmon classification. He was given upfront therapy with thalidomide at 200 mg/daily plus...
high dose dexamethasone. After 4 months of treatment, while in very good partial response from MM, the patient presented with headache, vomiting and fluctuating mental status. Fever appeared few hours later. The patient underwent lumbar puncture, CT and MRI; concomitantly repeated blood cultures were obtained. Cerebral spinal fluid (CSF) examination showed a WBC of 78/dL (85% neutrophils), glucose of 25 mg/dL, protein 240 mg/dL. Blood and CSF cultures were both positive for *Lysteria Monocytogenes*. MRI demonstrated multiple ipodense areas evolving in the following days in multiple brain abscesses. The patient started antibiotic therapy consisting of ampicillin and amikacin with disappearance of fever and slight improvement of neurologic signs; however, death occurred few weeks later from progressive encephalopathy. Case 2. CR, a 64-years-old male was diagnosed on January 2003 as having CLL in Rai stage IV. The patient received two courses of conventional dose chlorambucil (CLB) plus low dose steroids and achieved normal blood count with persistent lymphocytosis. Two weeks after the end of the second course, progressive confusion state, dysartria and fever occurred, needing admission to the hospital. CSF examination after lumbar puncture showed glucose 29 mg/dL, protein 276 mg/dL with a WBC of 4/dL; few days later, either blood or CSF culture resulted positive for *Lysteria Monocytogenes*. CT and MRI scan were normal. The patient received antibiotic therapy with ampicillin and amikacin without any neurologic improvement and died 10 days after the diagnosis of meningoencephalitis. Meningoencephalitis by *Lysteria Monocytogenes* is an infrequent infectious complication of patients with hematologic malignancies. Major predisposing factors are organ and stem cell transplantation, as well as treatment with fludarabine and/or corticosteroids. Nonetheless, in the present report the patient with MM had not received any cytotoxic agent, and the CLL one had only been given conventional doses of CLB and low doses corticosteroids, emphasizing that severe infectious episodes can occur in patients with LD in absence of aggressive therapeutic approaches. Our findings rise the question of prophylactic measures, including trimethoprim-sulphamethoxazole and/or dietary recommendations, in order to prevent *Lysteria* infections in patients with LD, independently from treatment with purine analogues or other cytotoxic agents.

**PU239**

MICROBIOLOGICAL SURVEILLANCE: ONE YEAR OBSERVATION IN A HEMATOLOGIC UNIT


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During the last year 464 admissions were performed in our unit. 26 were the patients admitted for therapy of newly diagnosed or relapsed acute leukemias. Due to the presence of febrile episodes during neutropenic phase, 162 were the hemoculture assays performed. Ninety-three were negative and 69 were positive (42,5%). The bacteria isolated are listed in Table 1. The large majority of them, 55/69 (79%), were GRAM+. According to the data of the literature we observed a prevalence of isolation of GRAM+ bacteria, even if the percentage of them is higher than those reported. These collected data allows us to map the prevalence of the bacteria that could infect our neutropenic patients, and could be useful to make a choice on prophylactic and empirical therapy grounds in our unit.

Table 1. Hemoculture positive findings (69/162).

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<td><em>Staphylococcus epidemidis</em></td>
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<td><em>Staphylococcus hemolyticus</em></td>
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<tr>
<td><em>Staphylococcus chromogenes</em></td>
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<td><em>Staphylococcus luteus</em></td>
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<td><em>Staphylococcus capitis</em></td>
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<tr>
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<tr>
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<td><em>Lactobacillus spp.</em></td>
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<td><em>Escherichia coli</em></td>
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<td><em>Bacteroides ovatus</em></td>
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PU240
CIPROFLOXACIN AND PIPERACILLIN/TAZOBACTAM IN ELDERLY NEUTROPENIC FEBRILE PATIENTS
Campioni L, Codari R, Ullori C, Giudici M E, Grandi AM
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Infections are the major cause of death among elderly people affected by hematological neoplasms. The rate of infections is closely related to the neutrophil count. Neutropenic patients with fever need an aggressive treatment with an empiric broad-spectrum antibiotic therapy, aimed at Gram-negative bacilli. Aminoglycosides remain an essential component of this treatment despite nephrotoxicity. A recent trial demonstrated that in patient with mean age 48 years, an association of β-lactam and quinolone is as safe and effective as β-lactam and aminoglycoside in neutropenic fever. Therefore we tested in elderly patients (＞60 years old) the effectiveness and nephrotoxicity of treatment with ciprofloxacin and piperacillin/tazobactam, selecting febrile neutropenic patients with hematological cancer. From January to March 2003 we treated 6 patients (1 male, 5 females; mean age 67 years, range 62-81 years) with hematological neoplasm (3 acute leukemia, 2 chronic lymphocyte leukemia, 1 multiple myeloma) with neutropenia (neutrophil count ＜ 0.5×10⁹ cell/L), and fever (＞38°C). All the patients were treated with ciprofloxacin 400 mg iv every 12 hours and piperacillin/tazobactam 4.5 gr iv every 8 hours. The infection site was found only in 4 patients (lung). Mean serum creatinine level before treatment was 0.91 mg/dL (range 0.67-1.32 mg/dL). Mean length of antibiotic treatment was 11 days (range 9-14). The therapy was effective in 4 patients with resolution of fever after 3 to 7 days of treatment. Serum creatinine remained unchanged in all the patients throughout the treatment period. In conclusion, our study demonstrated that in elderly patients antibiotic treatment with ciprofloxacin and piperacillin/tazobactam is effective (response rate 65%), moreover this treatment does not show any nephrotoxic effect. Obviously our results need to be confirmed by more extensive studies.

PU240bis
SEVERE FUNGAL INFECTIONS IN ACUTE MYELOID LEUKEMIA
Chierichini A, Luzi G, Ronci B, Monardo F, Nardelli S, Persiani M, Annino L
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The present induction strategy, in Acute Myeloid Leukemia (AML) while allowing the increase of the complete remission (CR) rate, it induces at the same time severe prolonged neutropenia, which may cause life-threatening infections. The onset of fungal infection during induction aplasia can prevent both chemotherapy continuation and performance of a successive PBSC transplant. We here report three patient cases with AML, who, during and after induction treatment, developed a Candida albicans spondilodiscitis(1) and Mucor spp. disseminated infection (2), respectively. Case #1: a 20 y-old woman, with a diagnosis of FAB M1 AML made in October 2001, was enrolled in the GIMEMA LAM 99 protocol achieving 1st CR in December 2001. In February 2002, after DNR+IDARA-C consolidation, she developed fever and radiculitis thus empiric treatment with glicopeptide and Fluconazole was started. Since spine pain persisted a NMR of lumbar rhachis was planned. NMR imaging evidenced the involvement of L5-S1 disk, thus, after the stop of screening for the HLA sibling AlloBMT, patient underwent neurosurgical biopsy. The diagnosis of Candida Albicans spondilodiscitis was performed both on histological and tissue culture grounds. Fluconazole therapy was continued for 8 weeks (t.d. 34 gr); but in April 2002, in spite of the clinical and radiological resolution of infection, patient relapsed and died one month later. Case #2: a 62 y-old man, was hospitalized in March 2002 because of a diagnosis of FAB M4 AMI; since he was refractory to 1st line (DNR+Ara-C) induction, he received a salvage therapy according to MEC (Mit+Etoposide+ID-ARA-C) schedule; after a prolonged (20d) severe neutropenia, patient achieved 1st CR, in May 2002. Before consolidation onset, patient showed disseminated necrotic cutaneous ulcer in the legs and left eye ophthalmitis which was suggestive of micotic infection; the biopsy of cutaneous lesions led the diagnosis of Mucor spp. panniculitis. Liposomal Amphotericine B treatment was started and continued for 16 weeks (t.d. 33gr); at the end of this therapy, patient, while persisting in hematological CR, achieved cure of cutaneous lesions and visus improvement. Disappointingly at the end of August patient had leukemia relapse, without any evidence of fungal lesions, thus he started palliative chemotherapy and continued anti-fungal treatment. Patient died 4 month later because of leukemia. Case #3: in July 2002, a 48 y-old man, with a previous 2 years lasting myelodysplasia, developed FAB M4 AMI. As concomitant diseases patient had severe cardiomiopathy and diabetes mellitus. During induction with low-dose Ara-C, he showed necrotic cellulitis involving nose, left cheek and soft palate. The lesion biopsy was carried out and the diagnosis of Mucor spp. infection was made. Patient received Liposomal Amphotericine B treatment for 12 weeks (t.d. 16gr) without any improvement. He died at the end of September in active disease, with face ravaged by infection. Antimicotic prophylaxis and empiric therapy represent a basic step of the entire treatment in AML since they help to prevent and to reduce the incidence.
of this heavy chemotherapy complication, but, up to date, there are no weapons to avoid the risk of fungal infections. Future approaches might include preemptive therapy on the basis of antigenemia dosage or other methods to an early screening of patients who must be treated with intensive schedules and, hence, at risk of prolonged severe neutropenia.

PU241
INVASIVE PULMONARY ASPERGILLOSIS IN LEUKEMIC PATIENTS
MANAGEMENT FOR INTENSIVE CHEMOTHERAPY COMPLETION IN THE ERA OF NEW ANTIFUNGAL THERAPY
U.O.C. Di Ematologia, A.O.R.N. A. Cardarelli, Naples, Italy

Invasive pulmonary aspergillosis (IPA) remains a life threatening complication in neutropenic patients. We report our experience in the therapeutic management of 8 patients with acute leukemia and IPA. Eight cases of aspergillus IPA (5 acute myeloid leukemia; 1 acute lymphoid leukemia and 2 myelodisplastic syndrome in evolution) were studied between June 2001 and June 2003. All patients were in induction treatment when IPA was diagnosed. Clinical signs included fever above 38 degrees and cough in all cases; chest pain in 3/8 cases (37.5%); hemoptysis in 3/8 cases (37.5%). Chest X ray showed one lesion in 5/8 patients (62.5%) and multiple lesions in 3/8 cases (37.5%). The diagnosis of IPA was established by tissue biopsy in 2/8 cases (25%); BAL in 2/8 cases (25%) and positive sputum in 4/8 cases (50%); thoracic computed tomographic (CT) scans were performed in all patients and showed one or multiple lesions with air crescent signs. Serological tests (aspergillus antigenemia) were positive in 4/8 cases (50%) late in the course of IPA. All patients were treated with i.v. Amphotericin-B (1 mg/kg/day) with subsequently itraconazole oral solution (neutrophy recovery). Pulmonary lobectomy was successfully combined with medical treatment in two cases (25%) after hematologic recovery and in one case was necessary (for amphotericin failure) successfully started with voriconazole therapy. After infection all patients were submitted to consolidation therapy and one patient died for invasive aspergillosis. Three patients were submitted to BMT (37.5%) 1 allo and 2 auto, with amphotericin or itraconazole oral solution as prophylaxis, and no also showed fungal infection recurrence. The overall survival in all patients is 80% with follow-up of 7-23 months. In conclusion a high index of suspicion and careful clinical and radiological examination are the key to identifying infected patients early and programming the following therapeutic steps. Above all in leukemia patients, prompt and aggressive administration of ant fungal agents seems to improve the outcome of invasive fungal disease and to permit intensive chemotherapy completion and transplant. In this patients the very important disease is acute leukemia and new ant fungal agents (voriconazolo; echinocandine etc.) permitted in this patients completion schedules for leukemic disease and transplantation to control fungal infections.

PU242
RECOVERY OF A NECROTIZING DERMATITIS IN REFRACTORY ACUTE MYELOID LEUKEMIA
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Cutaneous infections, particularly in neutropenic and immunocompromised patients, like leukemic patients, are difficult to manage and can rapidly become fatal if not treated promptly. We report on a 57-year-old man presented in July 1997 with essential thrombocythemia, then treated with an anticlumping therapy. In July 1998, the bone marrow and peripheral blood pictures showed an evolution to a myelodysplastic syndrome, of refractory anemia with excess of blasts type, then treated with occasional red blood cell infusions. In July 2000, a progression to a blast crisis was evident: Hb 5.7 g/dL, white blood cell count 5.6×10⁹/L with 3% neutrophils and 69% blasts, platelet count 9×10⁹/L. As the blasts were CD11 and CD33 positive an acute myeloid leukemia of M1 FAB subtype was diagnosed and a chemotherapeutic course (idarubicin and cytarabine) was done without a consistent remission. At that time, a wide necrotizing dermatitis (cm.16×9) appeared at right forearm, accompanied by intermittent mild fever. Hb 7.7 g/dL, WBC 1.8×10⁹/L with 2% neutrophils and 52% blasts, platelet count 10×10⁹/L. A skin biopsy specimen culture was positive for Staphylococcus epidermidis, whereas the blood culture and the serologic assay were negative. Antimicrobial susceptibility tests showed high sensitivity for vancomycin that was promptly administered intravenously (1g q12h). After 20 days of therapy the dermatitis resolved. Unfortunately, the patient died one month later by myocardial infarction. Our patient developed a serious Staphylococcus epidermidis necrotizing dermatitis, during a period of protracted neutropenia and when acute leukemia has became not responder to chemotherapy. It seems to be emphasized that, in neutropenic patients, appropriate laboratory tests, including culture of skin biopsy, may be necessary for the establishment of diagnosis of infection, even when serology or blood culture are negative. This approach can lead to recovery of life-threatening infections, in relatively short time, even in patients with refractory leukemic status.
Introduction. According to Italian Legislation, Nucleic Acid Amplification Testing (NAT) for searching HCV-RNA is performed on all blood donors. NAT's negativity is a necessary condition for biological validation of blood units. The purpose of this test is to detect HCV before the seroconversion, reducing the window period. The aim of this study is to compare NAT with MEIA (M icroparticle Enzyme Immuno Assay) and SIA (Strip Immuno blot Assay) establishing the benefits derived by the introduction of this new test. Materials and methods. The qualitative detection of antibodies to hepatitis C virus (anti-HCV) in serum has been performed by MEIA (Abbott Axsym System HCV 3.0). Repeatedly reactive samples have been subsequently tested with SIA (CHIRON RIBA HCV 3.0 SIA), which utilizes recombinant HCV-encoded antigens (c33c and N55) and synthetic HCV-encoded peptides (5-1 cle00 and c22) immobilized as individual bands onto test strips, to verify the presence of anti-HCV. NAT has been performed by transcription-mediated amplification assay (Chiron Procleix TMA HIV-1/HCV Assay). Results. In the period between June 2002 and June 2003, 10,088 blood units have been tested; 67 of them have been eliminated. Results are summarized in the table.

<table>
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<tr>
<th>Units (n.)</th>
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Conclusions: In literature, it's reported that NAT allows to detect donors affected with acute hepatitis before the seroconversion with a frequency of 1,600,000 blood donations. The discordance among NAT (negative) and SIA (positive) allows to underline infected subjects (8), but with no viral replication in act. We can affirm nothing about samples (16 + 8) with negative NAT and indeterminate SIA. Instead, the contemporaneous negativity of NAT and SIA points out donors (10 + 7) resulted false positive to MEIA screening. On the base of our results, NAT is advantageous not as screening, but because it is able to reduce the window period from 66 to 14 days. In conclusion, such test, associated to a rigorous pre-donation selection, based on accurate anamnnesis for risk factors and on sensitization of donors, is able to reduce the risk of a post-transfusion hepatitis C.
than the conventional form of amphotericin. However in some cases, high L-Amb doses or new agents, such as voriconazole or caspofungin must be considered.

PU245
PREVALENCE OF EXTRAHEPATIC MANIFESTATIONS IN 420 PATIENTS AFFECTED BY HEPATITIS C VIRUS INFECTIONS
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The aim of the study was to assess the prevalence of clinical and biological extraepatic manifestations of hepatitis C virus (HCV) infection. We analyzed the natural history of extraepatic manifestations of HCV infections by reviewing data recorded previously during the first visit of 420 consecutive patients with chronic HCV infection. The prevalence of hematological, neurological, nephrological, and dermatological manifestations, including the presence of autoantibodies and cryoglobulins was assessed. Extraepatic manifestations were found in 165 patients (37.5%) with chronic hepatitis C (more frequently in hepatic cirrhosis). Extraepatic illnesses were present to a greater extent in females. 146 patients (34%) had mixed cryoglobulinemia (MC) type III (87.6%) and type II IgM - k (12.4%). 30 patients (7.1%) affected with MC type II showed cryoglobulinemic syndrome with purpura of the lower extremities weakness and arthralgias. Peripheral neuropaty was found in 72 cases (17%), Raynaud phenomenon in 71 (17%) and sicca syndrome in 13 (6%). Elevated level of reumatoid factor were found in 112 cases (27%), hypocomplementemia in 58 (14%). Membranoproliferative cryoglobulinemic glomerulonephritis was found in 6 cases (1.4%). Low grade non Hodgkin's lymphoma (NHL), related to MC, were found in 18 cases (4.2%). MALT lymphoma of the stomach was found in 6 cases (1.4%), in two cases (0.5%) more a large cells NHL and CLL were found. Only one IgG - k myeloma was found. Monoclonal gammopathy was present in 29 patients (7%). Autoimmune thyroiditis was present in 6 cases (1.4%), porphyria cutanea tarda in 2 cases (0.5%) and lichen ruber planus in 6 cases (1.4%). Elevated ANA were present in 11 cases (2.6%), AMA in 3 (0.7%) and ASMA in 16 (3.8%). In conclusion, extrahepatic clinical manifestations are frequently observed in HCV patients. The most frequent hematologic abnormalities include mixed cryoglobulinemia, NHL and monoclonal gammopathy.

PU246
FATAL SCEDOSPORIUM APIOSPERMUM INFECTION AFTER NON-MYELOABLATIVE TRANSPLANTATION
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In the last years the use of fluconazole or amphotericin B for prophylaxis in patients receiving stem cell transplantation has a reduced mortality rates secondary to invasive infection from Candida albicans, but fungal infections still remain an important cause of morbidity and mortality in patients with hematologic malignancies and recipients of stem cell transplantation. Along with the most common fungal pathogens as Candida and Aspergillus species, there are an increase of infection caused by unusual opportunistic agent like Scedosporium Apioespurmum. Zygomycetes, Fusarium. We report a fatal case of S.Apiospermum in a patient receiving a non myeloablative allograft from an HLA-identical sibling. She was a 57-year-old woman affected by multiple myeloma IgA lambda, stage IIIA, xerocytosis. In the early phase after graft infusion she developed chest pain and fever and received empirical broad spectrum antibiotic therapy and intravenous amphotericin B oral solution from the start of conditioning. During the early phase after graft infusion she developed chest pain and fever and received empirical broad spectrum antibiotic therapy and intravenous liposomal amphotericin B (3 mg/kg) for persisting fever after 72 hour of hyperpyrexia. Chest XR showed a right lower lobe infiltrate. On day +14 the bone marrow aspiration showed a trilineage engraftment and neutrophil count was < 1000/L. Despite PMN recovery, patient's condition worsened and fever persisted. The chest XR showed multiple bilateral lung interstitial and alveolar infiltrates. Bronchoscopy showed a normal mucosa; bronchial lavage, only partially haematic, was collected and microbiological cultures were performed. Empirically echinocandin (50 mg/day after a first dose of 70 mg) was associated to liposomal amphotericin B. After few days the patient developed progressive hypoxic respiratory failure, mechanical ventilation was required, despite this she developed tension pneumothorax five days and she died on day +22. All blood cultures performed were sterile, but a mould grew out of bronchial lavage. The fungus was identified as Scedosporium Apioespurmum, an anamorphic state of Pseudallescheria Boydii. Autopsy revealed multiple lesions concentrated...
mainly in both lungs: in the upper right lobe a quite large nodular yellowish spongy-dry lesion with the characteristics of mycetoma was found. The brain presented multiple small hemorrhages in the white matter of cerebrum, cerebellum, pons and under the fourth ventricle floor. No other fungal localisation was detected. Disseminated S. apiospermum infection is a rare, but often fatal complication in immunocompromised hosts. It histologically and radiologically resembles invasive Aspergillus infection and early diagnostic is challenging often results in delay of appropriate medical therapy. Risk factors include allogeneic stem cell transplantation, T-cell depletion, tandem transplantation (autologous and allogeneic) and the use of ATG in the conditioning regimen. Early institution of antifungal treatment combining liposomal amphotericin B and echinocandin was not effective in this patient which progressed to respiratory failure and died.

**PU247**

**DISSEMINATED CRYPTOCOCCOSIS DIAGNOSED BY BONE MARROW EXAMINATION IN A CHRONIC LYMPHOCYTIC LEUKEMIA PATIENT**


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Cryptococcosis continues to be an important opportunistic infection in immunocompromised patients, particularly in those with acquired immunodeficiency syndrome. Disseminated cryptococcosis is also seen in hematologic patients, but there are few descriptions of bone marrow involvement in HIV negative cases. A 75 year-old man affected by Rai stage II B-CLL, previously treated with chlorambucil and prednisone with partial response, was admitted at our Department for fever (maximum 39 °C) of one week duration and progressive pancytopenia (leucocyte count 1,54×10⁹/L, 35% neutrophils, 55% lymphocytes, 5% monocytes, hemoglobin 8,7 g/dL, platelet count 8×10⁹/L). Other laboratory investigations showed a low CD4 count (CD4⁺ absolute value 279/mm³, with CD4/CD8 ratio 3.53), normal LDH, β-2 microglobulin 7,66 mg/L, γ globulin 22%. A bone marrow trephine biopsy revealed an interstitial infiltrate of small B-lymphoid cells (50-60% of cellularity) and numerous yeast-like encapsulated cells in a mucoid matrix with gelatinous appearance. There was also a granulomatous response with histiocytes and giant cells. The yeast cells stained black with Grocott’s methenamine silver stain and were surrounded by a capsule that stained brilliant magenta with mucicarmine. The diagnosis of bone marrow cryptococcosis was subsequently confirmed by aspirate culture, that led to Cryptococcus neoforms identification. The cryptococcal antigen titre detected by latex agglutination test was 1: 8192. A chest-X ray revealed a right upper-lobe pulmonary infiltrate, while a CT scan showed multiple hypodense intracerebral masses, without contrast enhancement, located in the occipital lobe, cerebellum and basal nuclei. Despite intravenous antifungal therapy with liposomal amphotericin B the patient presented a progressive impairment of cognitive functions and died for multiorgan failure after three weeks. Disseminated cryptococcosis is a rare but life-threatening fungal infection. Direct demonstration of yeast-like cells by bone marrow examination, confirmed by culture isolation and serology, could be very important in immunocompromised patient, particularly in case of febrile pancytopenia.

**PU248**

**SUCCESSFUL CONTROL OF PULMONARY AND CEREBRAL ASPERGILLOSIS WITH VORICONAZOLE IN A PATIENT AFFECTED BY ACUTE PROMYELOCYTIC LEUKEMIA**


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A 52-yo patient, diagnosed with AM L- M3 in January 2002, developed pulmonary and cerebral aspergillosis during neutropenia following induction treatment according to AIDA 2000 protocol. Pulmonary localisations were characterised by multiple nodules in both lungs, of diameter between 3 and 5 cm. A broncoalveolar lavage was performed, with the growth of aspergillus fumigatus. CNS localisation was characterised by two bilateral and symmetrical lesions, each about 3 cm of diameter, located in the parietal lobes. Diagnosis was confirmed through a brain biopsy which showed aspergillus hyphae. Colture gave growth to aspergillus fumigatus. Patient symptoms were characterised by fever, visual disturbances, slow ideation, ataxia, headache. Infection progressed during initial treatment with liposomal amphotericine. After fifteen days of treatment with amphotericine, compassionate-use voriconazole was started. At the same time the patient started to assume prophylactic treatment with diphenyldantoin. During the first 15 days of treatment with voriconazole the patient improved, and he was discharged with persistence of mild neurological symptoms. Over the following months on treatment the pulmonary lesions improved, and the two cerebral lesions remained stable. After the first cycle of chemotherapy, the patient still showed persistence of leukemia, with evidence of molecular disease: due to the infectious complication, he did not undergo any
other chemotherapy cycle, but continued only oral maintenance treatment with ATRA, 1 week q3w. This treatment was able to obtain the disappearance of PM L-RAR-α transcript from bone marrow. After 15 months of treatment with voriconazole at 400 mg po qd, patient is in excellent clinical conditions. He has no pulmonary symptoms. He does not present any neurological symptoms and he is continuing prophylactic treatment with dinoine. Biochemistry showed a persistent and important increase in colestatic indexes (GGT and ALP) without any elevation of bilirubin and with only mild elevations of AST and ALT. This report shows that prolonged treatment with voriconazole can successfully control a cerebral localisation of aspergillus infection. Again, it shows that prolonged treatment with voriconazole can be very well tolerated and can be administered without causing severe clinical adverse effects. Cotreatment with antiepileptic drugs is feasible. At the same time, it is interesting to note, as already shown by others, that successful control of AM-L-M3 can be obtained on the middle term by only one cycle of induction therapy, and subsequent maintenance with ATRA.

PU249
PROMPT RESOLUTION OF NASAL ASPERGILLOSIS WITH INTRA-NASAL INSTILLATION OF LIPOSOMAL AMPHOTERICIN-B (AMBISOME) AND GRANULOCYTE TRANSFUSIONS. A CASE REPORT
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Visceral fungal infections are a serious complication during chemotherapy in acute myeloid leukemia (AML) patients. At present, intravenous (i. v.) deoxicholate amphotericin B (fungizone) is the most frequently employed schedule for the treatment of these infections, and nephrotoxicity and infusion related chills are the principal adverse effects. The new liposomal formulation of amphotericin B (Ambisome, Gilead Science), both i. v. and directly intranasal. After some days, despite persisting neutropenia, the infection was resolved, without any toxicity. We think that intranasal administration of Ambisome might be useful for nasal aspergillosis in neutropenic AML patients.

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PU250
A CASE OF STAPHYLOCOCCAL TOXIC SHOCK SYNDROME WITH EXPANSION OF TCRαβ, CD4/CD8 DOUBLE NEGATIVE LYMPHOCYTES
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A 55-year-old woman presented with toxic shock syndrome (TSS) due to Staphylococcus Hemolyticus. During the course of the disease a significant leukocytosis (20×10⁹/L) with lymphocytosis (16×10⁹/L) appeared and, after flow cytometry evaluation by means of MoAbs, a high number (7×10⁹/L) of TcR αβ⁺CD4/CD8 double negative (DN) T-lymphocytes was observed both in bone marrow and in peripheral blood samples. The immunophenotypic properties of circulating lymphocytes were evaluated during the course of TSS, which was treated successfully. The percentage of DN T-lymphocytes decreased slowly and two weeks after clinical remission a significant number of DN T-lymphocytes (1.05×10⁹/L) was still observed. The complete correction of the altered lymphocyte immunophenotype was observed only one month after recovery from TSS, when the WBC differential count was PMN 74%, lymphocytes 23%, mono-
cytes 3% and the immunophenotype was CD3:75%, CD4:40%, CD8:35%, CD5:76%, CD19:15%, CD56:10%. The immunophenotype of circulating and bone marrow lymphocytes was studied also during a phase of an aspecific febrile episode observed two months after recovery, but a subset of CD4/CD8 double negative T-lymphocytes was not found. Staphylococcal TTS is generally caused by production of a staphylococcal exotoxin named TSST-1, which acts as a superantigen, binds to both MHC class II molecules and specific Vβ regions of the T-cell receptor and leads to the activation of both antigen-presenting cells and T lymphocytes. As a consequence, production of pro-inflammatory cytokines and T cell proliferation occur with: release of IL-1, γ-IFN and TNF by monocytes, powerful proliferative effects on T cells, profound state of T-cell unresponsiveness. Howev-er, after an extensive Medline review, this is the first case of TSS characterized by expansion of DN T-lymphocytes. A very small subset of TcR αβ+DN T cells can be found in normal bone marrow, liver, thymus, skin. These cells show peculiar immune regulatory properties and can increase in particular autoimmune diseases. Our finding may represent a peculiar effect of lymphocyte stimulation by the staphylococcal exotoxin and suggests that further studies should be carried out in TSS, aimed to achieve a deeper knowledge of the lymphocyte immunophenotype in this peculiar disease.

PU251
ACUTE HEPATITIS C VIRUS INFECTION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES
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*Clinica delle Malattie Infettive; *UO e Cattedra di Ematologia, AO Careggi, Università degli Studi di Firenze, Italy

Patients with hematologic disease require repeated blood and platelet transfusions. Therefore these subjects are exposed to a significant risk of acquiring post-transfusion hepatitis C virus (HCV) infection. However immunosuppression may cause a delay in seroconversion and a correct interpretation of clinical and biochemical data can be difficult. Our study was aimed at describing the clinical course, the pattern of seroconversion, the behaviour of HCV-RNA and its correlation with HCV core antigen levels in four consecutive patients with hematologic malignancies and acute HCV infection. Patients and methods: Four patients with hematologic malignancies (3 pts with acute myeloid leukemia, one with multiple myeloma) and acute HCV infection were studied. All patients were off-therapy for the underlying malignancy and were not cytopenic. Clinical and biochemical examination was performed regularly and sera obtained at 7-28 days interval (mean 19 days) during the acute phase were analysed for the presence of HCV antibod-ies (ELISA), HCV RNA (PCR), and HCV core antigen (ELISA). Results: Acute HCV hepatitis was characterized by serum aminotransferase (AST, ALT) levels rise (mean ALT value±SD at onset 266±63 U/L). Jaundice was present in 3/4 cases with a mean bilirubin peak level of 17.7±9.2 mg/dL. Anti-HCV was detected at onset in one patient, while it was negative in the remaining 3 cases. During the acute disease it became detectable in one patient after 2 weeks, in one patient remained undetectable after 15 months and in one patients was still undetectable 9 weeks after onset of acute hepatitis when the patient died for sepsis. HCV-RNA was readily detected with a mean value of 751.666±972.911 IU/mL, and remained detectable with variable fluctuations for the entire duration of follow-up (9-15 months) indicating progression of the infection to chronicity. HCV genotype was identified in 3 patients and it was 1b in all cases. ALT levels were persistently raised, showing in only one patient transient fluctuations to normal values during a 12 weeks period. HCV core antigen was positive in all sera with a mean value of 84±106 pg/mL, concomitantly to HCV-RNA and its levels correlated significantly with the HCV RNA serum levels (Pearson’s test R=0.006, p<0.001). Conclusions: Acute HCV infection represents still a risk in patients with hematologic diseases requiring blood transfusions, and the prevalent genotype seems to be 1b. Progression of acute HCV infection to chronicity is commonly observed in such patients. The delay or the lack of seroconversion during the acute disease underlines the need for HCV RNA detection in any patient with acute rise of ALT levels. HCV core antigen testing may prove a useful, cost-effective diagnostic tool for screening and follow up in a setting where risk of infection and toxic injury of the liver may represent a diagnostic challenge.

PU252
PROBABLE ASPERGILLOSIS WITH LUNG AND BRAIN STEM INVOLVEMENT. FAVORABLE OUTCOME WITH VORICONAZOLE THERAPY
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Aspergillosis is the more common infection by filamentous fungi in hematologic malignancies. A diagnosis of proven aspergillosis, according to EORTC/M5G classification, requests positive histo-cytopathologic examination or positive culture of the affected site. A positive test for aspergillus antigen supports only a probable diagnosis. In a recent study of GIM EM A, brain aspergillosis was diagnosed in 37/391 patients, in 35 the diagnosis was confirmed at autopsy, and only in 2 it was made from biopsy. 36 patients died (Pagano L Haematologica 2001 86:862). With the new antifungal therapies, brain aspergillosis could no more be an
invariably fatal disease. In order to study this very serious complication perhaps more flexible diagnostic criteria are needed, because these patients are usually too ill to undergo invasive tests. Otherwise the clinical applicability of the EORTC/MSG classification has been recently discussed (Martino S Ann Hematol 2003;82:80-2)

2 A 65-year woman was admitted in our department with myeloid acute leukemia M4. She had broncho-pneumonic infection of the lower left lobe. She was treated with tazobactam 12 g and levofloxacian 500 mg, fluconazole 200 and then 600 mg. ICE-chemotherapy was started three days after the admission, followed by filgrastim 300 µg. Neutropenia <100 neutrophils/mm³ lasted from the fifth to the eighteenth day from the beginning of chemotherapy. The patient was very ill with profound asthenia, moderate dyspnea and profound asthenia, moderate dyspnea and hyposiemia, dry cough, remitting fever up to 40°C, bibasilar rales. Blood cultures were positive for Staphylococcus epidermidis and Enterococcus faecium. Meropenem 3 g, teicoplanine 400mg, amikacine 1gr, and cefazidime 6gr were also used. Cytomegalovirus antigen/DNA in the blood and Pneumocystis carinii in the sputum were absent. On the sixth day amphotericin-B was started, but the conditions remained very serious also after the rise of neutrophils. TC-scan of the chest showed multiple diffuse nodules. From the fifteenth day dizziness and mild obnubilation and from the eighteenth day left hemiparesis developed. Head Tc-scan didn't show any lesion. RMN demonstrated diffuse signal alteration of the of brain stem at the pontal level. Desametazone 8mg day was introduced. Platelet-test for galactomannan antigen of Aspergillus, which was first negative became positive in two determinations (eighteenth and twentieth day). The introduction of liposomal-amphotericin-B didn't lead to any improvement. Twenty days after the beginning of chemotherapy we suspended amphotericin-B, for the lack of results and also for intractable hypokaliemia and started voriconazole 200 mg twice iv. After 2 days fewer subsided, obnubilation, cough and dyspnea improved. Biopsy showed complete remission. She had a second course of chemotherapy without complications. There is now a nearly complete remission of neuropsychiatric features and immunohistochemical assays were highly suggestive of CMV colitis. Positivization of CMV antigenaemia (2 nuclei per 200 000 cells) was detect-ed because of the neutropenic state of our patient, we opted for a three-week treatment with Foscarnet, obtaining a rapid resolution of gastrointestinal symptoms and persistent negativization of CMV antigenaemia. Further evaluation showed a significant improvement of the endoscopic and histological findings. This is the first reported case of CMV colitis occurred during standard induction chemotherapy for ALL. We discuss the case and focus on the problematic issues regarding diagnostic process for gastrointestinal CMV disease and antiviral treatment options in ALL patients diagnosed with CMV colitis.
A PATIENT WITH ACUTE MYELOID LEUKEMIA
SUCCESSFUL OUTCOME OF A DISSEMINATED FUSARIOSES IN
Fusarium species are common soil saprophytes. In
immunocompromised patients they are actually identified as
an emerging infective agents. In these patients, this
filamentous fungus causes aggressive infections with
a very poor prognosis and an high mortality rate,
despite antifungal therapy. We report the case of a
young patient with acute myeloid leukemia (AML) who
presented a disseminated fusariosis. A 25-year-old
woman was admitted for acute myeloblastic leukemia
(FAB M0) and underwent induction therapy including
idarubicin, etoposide and cytarabine. Four weeks later,
a bone marrow aspirate demonstrated complete infiltra-
tion by leukemic cells. The patient was then treated with
high dose cytarabine and mitoxantrone as salvage ther-
apy. Two weeks later (8 weeks of neutrophil counts
below 0.5 x 10^9/L) the patient developed disseminated
skin eruption consisting of several nodules with differ-
ent aspects: papuloerythematous, hemorrhagic, with
brim, with central necrosis (especially on the limbs).
Arthralgia and swelling of the arms, hand and legs with
functional impairment and severe asthenia were also
present. After two days she developed fever and a pro-
gression of the cutaneous picture was observed, with
diffuse, severe muscle and joint pain. Fusarium solani
was isolated from skin biopsy and multiple blood cul-
tures. The patient was treated with liposomal amphi-
tericin B plus flucytosine. After the first days of treat-
ment general conditions worsened. Because of persistent
and severe neutropenia she was also infused with nor-
am donor granulocytes from apheresis. The treatment
obtained a slow improvement, with disappearance of
the fever, reduction of the pain and of the skin lesions.
The hematologic recovery was complete and the marrow
aspirate showed complete remission. She started a phys-
thoerapeutic program and was discharged from the
hospital 3 months after her admission. According with
the literature, this report confirms that invasive fusar-
iosis is a rare and life-threatening complication but a
combination of systemic antifungal agents and granu-
locytes from single donor apheresis can improve the out-
come in neutropenic patients.

Introduction: Bacterial infection is a major cause of
mortality in patients with cancer and prolonged neu-
tropenia. In spite of several clinical studies and of an
extensive use of quinolones, the beneficial effects of oral
prophylaxis in neutropenic patients remain controversial,
because of the increasing frequency of Gram positive
infections and of the emergent resistance of Gram neg-
ative pathogens. Design and Methods: We analyzed the
episodic of bacteremia during febrile neutropenia
occurred among adult patients followed in our Hema-
tologic Unit from January 2000 to December 2002. We
evaluated the therapeutic phases with high infective
risk: induction, consolidation and savage treatment for
acute leukemias and high dose chemotherapy and
autologous hematopoietic stem cell transplantation
(HSCT) for acute leukemias and lymphoproliferative
disorders. Results: During this period we followed 128
high infective risk phases: 35 in the first year (11 autol-
ogous HSCT and 24 acute leukemias), 43 in the second
year (7 HSCT and 36 acute leukemias) and 50 in the third
(13 HSCT and 37 leukemias). All the patients were on
prophylaxis with quinolones (Ciprofloxacin 500 mg twice
daily or Ciprofloxacin 500 mg daily × os) since the start
of chemotherapy until granulocytic recovery (> 500/mm³).
The incidence of febrile neutropenia and the rate of bacteraemia were similar: 12 (34%), 15 (35%) and
14 (28%) during 2000, 2001 and 2002 respectively
during 2000, 2001 and 2002. On the contrary, blood cul-
tures gave different results: Gram positive microorgan-
isms were responsible for 83% and 80% of the bac-
teria episodes during the years 2000 and 2001, while
during the last year the Gram negative pathogens rep-
resented the 70% of the isolated from blood (p=0.003).
In particular Escherichia Coli resistant to ciprofloxacin
and levofloxacin was isolated in 8/10 cases (80%). All
the patient but one with Escherichia Coli sepsis, were
previously treated with quinolones. Conclusions:
Although the good clinical efficacy and compliance of
quinolone prophylaxis have been clearly established, ever
increasing number of reports point out the frequent
occurrence of Gram negative bacteria resistant to
quinolones, especially Escherichia Coli. The experience of
our hospital, with a quick change in the ethiology of
bacteremia and the prevalence of quinolones resistant
enterobacteria suggests that the fluoroquinolone pro-
phylaxis should be carefully discussed in the future.
PAIN SYNDROMES IN HEMATOLOGIC MALIGNANCIES

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Patients with hematologic malignancies may experience many different types of pain, related to the disease itself, the treatments, debility or to other illness. Pain syndromes in this setting are the least studied and under-recognised and under-treated. Further studies aimed to improve the pain management in the hematologic setting are awaited.

Quality of Life, Pain Therapy and Familial Assistance

PU256

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Peripheral polyneuropathies (PNP) are the presenting feature in about 15% of patients with AL Amyloidosis, may developing at some point of clinical course in up to 35% of cases. We report on a patient presenting a PPN-associated AL Amyloidosis. Case report: A 58 years old male was admitted to our observation on April 1998 because of a paraprotein level of 3,05 g/dL. A polichemotherapy, including melphalan, dexamethasone and intravenous zolendronate, was started. Pain, evaluated with a numerical rating scale (NRS), given a rating of 1 to 10, was turning out unrelived by the specific therapy and worsened (NRS = 8) after the end of the first MD cycle, although the administration of tramadol and paracetamol. He received a symptomatic therapy with gabapentin, at progressively increasing doses until to 1600 mg/day, combined with slow release tramadol (100 mg bid). In the following days, a complete pain relief and the disappearance of the parestesias was recorded. After the completion of the MD chemoterapy, that induced a good response, the patients presented a symmetric numbness and dysesthesias of the upper limbs, risen quickly and taken from important strength deficits. An electomyography showed an axonal sensorimotor neuropathy of the lower limbs. The anti-nerve antibodies (anti MAG) were negative. An amyloid PPN was suspected and patient was re-evaluated. A 25% plasma cells was found in bone marrow; the PUF revealed the presence of amiloid substance; an Rx ray survey evidenced diffuse skeletal osteolitic lesions; the paraprotein level was 3,05 g/dL. A polichemotherapy, including melphalan, dexamethasone and intravenous zolendronate, was started. Pain, evaluated with a numerical rating scale (NRS), given a rating of 1 to 10, was turning out unrelived by the specific therapy and worsened (NRS = 8) after the end of the first MD cycle, although the administration of tramadol and paracetamol. He received a symptomatic therapy with gabapentin, at progressively increasing doses until to 1600 mg/day, combined with slow release tramadol (100 mg bid). In the following days, a complete pain relief and the disappearance of the parestesias was recorded. After the completion of the MD chemoterapy, that induced a good response, the patients presented a symmetric numbness and dysesthesias of the upper limbs, risen quickly and taken from important strength deficits. A neurological re-assessment revealed a diffuse and progressive sensory-motor PNP, findings consistent with a diagnosis of mononeuritis complex, in which symptoms evolve at different time and to different degrees in different nerves. To date, patient is on gabapentin and tramadol; he presents a stably maintained remission of the MM and is managed with monthly infusion of zolendronate associated with dexamethasone. Nevertheless, he presents a progressive strength deficits and important functional deterioration. AL Amyloidosis may lead to a range of PNP as well as entrapment neuropathies due to increased volume of nerve or supporting tissue secondary to amyloid deposition. The polineuropathic forms are due to focal amyloid deposition within the nerve sheaths, that induce a direct toxic effect and a vascular insufficiency. In typical cases small myelinated and unmyelinated nerve fibres are affected leading to a chronic slowly progressive axonal PNP that is distal and usually symmetric. Sensory changes, such as burning, aching and lancinating pain, are usually the first symptoms. Atypical patterns of amyloid PNP include motor-neuron disease with demyelinating features. Dysautonomic symptoms is usually prominent but they may be a late manifestation of disorders. The sural nerve biopsy is the recommended diagnostic procedure but it is unidiagnostic in many cases, given that the amyloid deposits can be focal, and requires a specialised skills to be performed. The management of these patients is a challenging concern, being no specific option treatments and the therapies-directed to the underlying disease can leading only few functional and clinical improvements. Melphalan combined with prednisone prolongs survival in a small proportion of patients for several years, but the treatment has little effect on the neuropathy. In recent papers, high dose (HD) chemotherapy followed by autologous stem-cell transplantation (ASCT) has improved or stabilised the condition of a few patients. Pharmacological treatment of neuropathic pain has traditionally based on antidepressants, antiepileptic and membrane stabilising drugs, while the use of opiates is still discussed. Recently, gabapentin has been reported as effective and well tolerated in the management of neuropathic pain. Tramadol provides peripheral and central analgesic effects, exerting his action by binding the mu-opioid receptors and by inhibiting serotonin and nor-epinephrine reuptake. It is considered in patients with nociceptive mild to moderately severe pain and is suggested also in treating those of neuropathic origin. Moving from these premises, we have successfully adopted this therapeutical association in the symptomatic management of our patients. Given the limited management of the amyloid PNP and the poor prognosis of these suffering patients, the early diagnosis is so far the most important goal, permitting an effective intervention, including the HD chemotherapy with ASCT in able candidate, before the nerve damage occur.
The bone is the most common source of painful disease-related syndromes in patients with hematologic malignancies. The prominent painful syndromes originating from the bone are caused and maintained by partially known biological and neurophysiological mechanisms. Among the deep somatic structures, the periosteum has the lowest pain threshold and is a frequent site of pain. A plexus provided by the nerve endings of thinly myelinated A-delta fibers, responding to movements, and by the unmyelinated C fibers, sensitive to thermal chemical and intense mechanical stimuli, richly supplies it. The mineralized bone also receives the free nerve endings of the same fibers, whereas cortex and bone marrow (BM) are not pain-sensitive. The tumour bone involvement, through the induction of cyclooxygenase (COX)-2 mediated by interleukin (IL)-1β, sustains the release of prostanoids, mainly prostaglandin E2 (PGE2), which sensitise peripheral nociceptor terminals, producing localized pain hypersensitivity. In the central nervous system (CNS), given the widespread of COX-2 expression in this site and the elevated PGE2 levels in the cerebrospinal fluid, PGE2-induced functional modifications cause centrally generated pain hypersensitivity, lowering the patient’s pain threshold. So, without early effective therapy, peripheral and central sensitization reciprocally contribute to sustaining and chronically maintaining pain. Cancer pain rat and mouse models (Monthy et al.) have demonstrated that an early central sensitisation (dramatic rise of astrocytes in the spinal cord and CNS) follows tumor implant. This sensitisation results from an early cross talk between the site of tumour growth and CNS. In this process (neuroinflammation) the immune activation play a crucial role suggesting a continuum between nociceptive (opioid responsive) and neuropathic (opioid non responsive) pain. These findings stress the need for early modulation of peripheral nociception as prevention of opioids poor or responsive-ness. According to the pattern of the bone involvement, different painful syndromes, sometimes overlapping, may be recorded. Patients with multiple myeloma (MM) often present with lytic skeletal lesions, pathological fractures and vertebral collapse. The constitutive release by myeloma cells of osteoclasts-activating factors, mainly the TNF-β and IL-1, leads to microfractures, occurring most frequently in lumbar spine, cranial bone, thoracic vertebrae and ribs, which may be complicated by muscle spasm, nerves infiltration and vertebral collapse. Osteolysis, although rarely, has also been reported in patients with non-Hodgkin’s lymphoma, hairy cell leukemia, adult T-cell leukemia and malignant chronic myeloid disorders. In acute leukemias, although local osteolysis has been rarely reported, significant osteoarticular changes may be found in a remarkable proportion of patients, above all in children. In patients with BM involvement, typically occurring in leukemias, the mechanism of pain may be related to the expansion of the hematopoietic matrix within the rigid-walled space, causing intraosseus hypertension, impairment of blood supply and distension of the periosteum. So, a patient with skeletal lesions suffer from a localized and sometimes irradiated nociceptive continuous pain at rest, complicated sometimes by neuropathic symptoms (mixed pain), and incident pain, evoked by movements, while the BM expansion-related pain is diffuse and continuously migrating, and may be associated with neuropathic signs, becoming excruciating. Management of malignant bone pain is guided by two principles: treatment of the underlying condition with tumour-directed therapies, radiotherapy or orthopaedic devices and surgery, and strategies designed to relieve pain irrespective of cause (analgesics). The mainstay of treatment in patients with lytic lesions is radiotherapy that can allow quick pain relief and reconstitution of bone continuity. In patients with multifocal lesions, the radiotherapy may be evaluated on case-to-case basis. Orthopedic devices are extensively applied, while surgery (percutaneous vertebro- and kyphoplasty, intramedullary fixation of fractures of long bones) may be reserved for selected cases. The adjuvant drugs play an important role in pain management. Bisphosphonates provide a meaningful therapeutic effect on skeletal disease, reducing pain and vertebral fractures, as demonstrated in patients with MM. Intravenous zoledronic acid, requiring a significantly shorter infusion time, has recently been shown to be as effective as intravenous pamidronate. Pain management is a clinical challenge, considering that many variables, such as concomitant severe neutropenia and thrombocytopenia, are likely to be involved. The use of non steroidal anti-inflammato-ry drugs, highly effective in relieving bone pain arising from tumor skeletal involvement, being able to inhibit the prostaglandin synthesis, are currently avoided, although the development of the novel selective COX2 inhibitors, which have been reported to have less effect on constitutive renal, gastric and platelet COX1 activities, may require specifically addressed studies on their safety in hematologic patients. Steroids can indirectly provide pain relief, blocking COX-2 enzymes, reducing peritumoral edema and BM hypertension. Analgesic drugs may be supplemented with antineoplastic chemotherapy, palliative radiotherapy and adjuvant agents as appropriate. According to the World Health Organisation three-step analgesic ladder, depending on the severity of the pain, opiates, administered at the appropriate doses, are the mainstays of therapy, being able to control bone pain at rest in most of cases. Given that breakthrough and acute incidental pain, transmitted by the opiate poorly responsive A-δ fibers, is difficult to completely relieve, a rescue dose of a rapid-
ly short acting drug, such as oral tramadol or morphine, may be available on demand or for pre-emptive analgesia in case of intentional movements or programmed procedures. So far, few studies have specifically addressed this issue. Further data are needed to improve our knowledge and guidelines for the best management of these patients are warranted.

PU259
PAINFUL SYNDROMES IN MULTIPLE MYELOMA: THE EXPERIENCE OF A SINGLE CENTER AND A BRIEF OVERVIEW
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Patients with multiple myeloma (MM) may experience several pain syndromes, associated with the disease itself, the treatments or with indirectly related conditions, such as the debility and the infections. Skeletal pain, associated with lytic lesions, pathological fractures and vertebral collapse, is the more frequent source of morbidity for patients with MM. We report on the pain syndromes recorded in the series of MM patients, followed in our Centre in the last ten years, together a brief summary on the current recommendation for management. There were 106 patients (M: 47, F: 49), with a median age of 69 (42-90) years. Out of these, 93 (88 percent) presented at least one pain syndromes for a total of 125. The underlying mechanisms were identified as follows: 101 (81%) osteolysis, 76% (76%) arthrosis, 5 (4%) painful plasmocytomas and 12 (9%) neuropathies. Antineoplastic treatments, radiotherapy, orthopaedic devices and analgesics were applied as required. The painful syndromes arising from the bone are caused and maintained by biological and neurophysiological responsible mechanisms, which in part remain poorly understood. The constitutive release by myeloma cells of osteoclasts-activating factors, mainly the TNF-β and interleukin (IL)-1, leads to micro fractures, occurring more frequently in lumbar spines, cranial bone, thoracic vertebrae and ribs, that may be complicated by muscle spasm, nerves infiltration and by vertebral collapse. Therefore, the induction of the cyclooxygenase (COX)-2, mediated by IL-1β, sustains the release of prostaglandin E2 (PGE2), which sensitises the peripheral nociceptors. In the central nervous system (CNS), where the COX-2 is widely expressed, the PGE2-induced functional modifications that origin a centrally generated pain hypersensitivity, lowering the painful threshold. So, if the continuous painful stimuli have not been interrupted by an effective treatment, peripheral and central sensitisations reciprocally contribute to sustaining and chronically maintaining the pain. Therefore, it has recently been suggested that plasma cells may express neurotrophin, as the brain-derived neurotrophic factor (BDNF), know to cause hyperalgesia by increasing the activity of nociceptive neurons, amplifying the MM skeletal pain (Y Li, 2003, Multiple Myeloma, 9th International Workshop). Skeletal lesions give rise to a localized and variably irradiated nociceptive continuous pain at rest and to an incident pain, evoked by the movements. In case of painful bony lesions, especially if prone to fracture, the radiotherapy is the cornerstone of the treatment, quickly allowing effective pain relief even of pain refractory to causal anti-MM and analgesic therapies. The pharmacological pain management in these patients, traditionally avoided the use of non steroidal anti-inflammatory drugs, although there is a lack of data on the safety of the novel selective COX2 inhibitors, suggested as able to sparing constitutive renal, gastric and platelet COX1 activities. Steroids can provide indirect pain relief, blocking the COX-2 enzymes, reducing the peritumoral edema and resolving critical compression exerting by paravertebral occupying-space masses. In patients with solitary or MM of the spine presenting refractory spinal pain, percutaneous vertebro- and kyphoplasty are safe, feasible and effective methods. Neuropathic pain is commonly associated with peripheral neuropathy that complicated about of 10% of MM patients. Analgesic drugs may be supplemented with antineoplastic chemotherapy, palliative radiotherapy and adjuvant agents as appropriate. According to the World Health Organisation three-step analgesic ladder, depending on the severity of the pain, opiates are the mainstay of therapy. Given that breakthrough and acute incidental pain is difficult to completely relieve, a rescue dose of a rapidly short acting drug, as oral tramadol or morphine, may be available on demand or for pre-emptive analgesia in case of intentional movements or programmed procedures. The bisphosphonates exert an important role in the MM management, providing a meaningful therapeutic effect to the bone pain and to skeletal disease. Intravenous zoledronic acid, that requires a significantly shorter infus- sion time, has recently been shown to be as effective as intravenous pamidronate. There are no specific option treatments for patients with neuropathies. The symptomatic approach is based on the antidepressants, antiepileptic and membrane stabilizing drugs. In our recent experience, a significant pain relief has been achieved by gabapentin in escalating dose associated with a strong opiate or with tramadol. Although therapeutic strategy for MM is moving fast to curative intents, the pain control and the patient's quality of life remain two important goals for most patients, requiring continuous efforts in improving the multidisciplinary skills.
The Day Hospital for onco-hematological patients of Milan's Ospedale Maggiore has been equipped with an internally developed ad hoc information system designed to manage its clinical and administrative activities. The system is capable of effectively supporting booking and reception activities, the compilation of SDOs, the computer-aided production of clinical charts and discharge letters, and integration with the Health Information System. The booking function allows bookings to be made with various operating units using the same application environment; the user interface has been designed in such a way as to allow the simultaneous, rapid and effective use of the system by operators belonging to different units. Every reservation can be associated with detailed information concerning the treatment to be administered. By means of personalisable and facilitated entry keys, the system allows it possible to specify all of the services to be provided to individual patients: this is particularly useful in the case of oncological therapies involving entire treatment cycles, which can be loaded in the system easily and quickly. The different units can dynamically and completely autonomously configure their assigned resources. Every operator of the system can monitor the trend of booking activities insofar as the following information is always available: the total number of patients with appointments during the day and the number of patients booked for every single bed/armchair. The system is capable of handling urgencies and providing a summary view of the status of reservations and activities on an annual basis. The system is completely integrated with the hospital's administrative application: at the end of each DH stay, it is possible to confirm the services provided and automatically record the movement in the hospital archives for subsequent processing. The system includes a tool for the computer-aided compilation of SDO which allows the completion of the diagnosis and procedure codes for every movement actually recorded, thus closing the DH admission file and making the record available for forwarding to the Regional authorities. Finally, using the information entered at the time of the booking, the written notes made by the medical staff during examinations, and the data taken from the reports of the diagnostic investigations made by the hospital's operating units, the system allows the computer-aided production of the discharge letters concluding every DH admission. Field tests have shown that, by overcoming the need for the manual recomposition of clinical documents, working times have been reduced and the quality of the documents has decidedly improved. The application was developed using the object-based development tool Visual FoxPro v. 6.0. All of the information is resident on the hospital's Oracle v. 8.1.7 database, but the application can be supported by any of the latest-generation databases.

PU261
Not published.

Supportive Therapy

PU262
EVALUATION AND PSYCHOLOGICAL SUPPORT IN ONCOHEMATOLOGICAL PATIENTS IN A BONE MARROW TRANSPLANTATION UNIT
Terruzzi E, Amà A, Montesano R, Parma M, Todisco E, Carraro M, Rossini F, Pogliani EM
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Recovery from allogeneic and autologous stem cell transplantsations requires a long-term course, often accompanied by acute morbidity, which includes various distressing physical symptoms. The assessment and procedures performed by medical equipment includes: i) patient's clinical and psychological assessment using a psycho-diagnostic interview (defensive attitude and/or adaptation to disease and treatment protocol); ii) implementation of psychological support (individual weekly interview taking about 30 minutes) during hospitalisation; iii) analysis of quality of life with a questionnaire EORTC-QLQ-C-30 and compilation of a clinical and psychological record with observations obtained during multidisciplinary (physicians, nurses and psycho-oncologists) evaluation of patient. We have identified three areas of psychosocial morbidity: a) psychological problems (fears about the future, sense of loss of control, anxiety and depression); b) physical problems; c) community reintegration problems. In our experience we have obtained: a) more integration among patient’s needs; b) more sharing, into the equipe, of the emotional experience related to the care role; c) more stimulus and/or exchange between different professional roles.
The aim of the present work was to verify our experience in current quality control (QC) of 20 platelet (PLT) concentrates (single donor products) by apheresis with cell separators (Autopheresis Baxter and MCS Plus Hemometrics). We studied fragments and PLT clumps 24 hours after the procedure using an automated analyzer Dasit XE - 2100. These were the principal parameters observed: Total Platelet Count, Large Platelets (P-LCR), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), PLT fragments and Q-Flags Alarm, PLT area in the PLT-O scattergram optical platelet count. Platelets are usually counted by an impedance system (PLT-I); in case of PLT-I channel inaccuracy and the presence of cell fragments, an optical count automatically (PLT-O, HydroDynamic Focusing-HDF) starts, giving more reliability in the presence of large platelets or microcytosis. Results: PLT count 996.81×10^4/µL (771-1272); P-LCR presence 16.18% (9.3-23.1); MPV 8.66 fL (7.7-9.6); PDW 9.5 (7.7-11.0), fragments alarm, discrete presence in all concentrates; PLT clumps by Q-Flags, 18 samples out of 20 and 2 negative (range 0-300, standard setting 0-100). Large platelets and fragments size activation as a result of stress, cause platelet clumps resulting in automated measures and are a limited supply of platelets properly managed. At the same time little attention has been paid to the appropriate dose, number and over all to functional activity of platelets transfused. The present work and these simple data provided by an automated hemocytometer are proposed markers of audit criteria for platelet transfusion and to monitor physicians' behavior; the intent of instrument-generated flags is limited to the identification of specimens containing abnormal cells, subject to review by qualified laboratory personnel.

PU264
SUCCESSFUL TREATMENT OF AUTOIMMUNE DISEASE WITH ANTI-CD20 MONOCLONAL ANTIBODY (RITUXIMAB):
A CASE OF RECALCITRANT PEMPHIGUS VULGARIS
Virgolini L, Marzocchi V, Mazzaro C
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Pemphigus vulgaris (PV) is an autoimmune disease of the skin and mucous membranes and is potentially life-threatening because of the presence of flaccid bullae that rupture and leave erosions and scars. The use of systemic corticosteroids is the most effective therapeutic regimen but its prolonged administration may lead to serious side effects. For many patients it is necessary to add immunosuppressive agents or use chemotherapy to achieve remission; sometimes all treatments are unsuccessful. The chimeric human-mouse monoclonal antibody Rituximab reacts specifically with the CD20 antigen and induces a marked and prolonged systemic B cell depletion. Its use in B cell Non Hodgkin lymphomas is consolidated but the strong reduction in antibody production has encouraged the utilization in auto-immune diseases as well. M M, a 53-year old female, had a well documented diagnosis of PV. She was treated for many years with corticosteroids, pulse doses of methotrexate and in the last year required 60 mg/day of methylprednisolone and 100 mg/day of cyclophosphamide to obtain an unsatisfactory control of the disease. On June 2002 a rituximab treatment at a scheduled dose of 375 mg/m²/ week was started and completed in four weeks. The treatment was well tolerated and no side effects were observed. Three months after the end of the therapy we observed a complete healing of cutaneous lesions. One year after the patient is still in complete remission. This case confirms that rituximab is appropriate, effective and safe in the treatment of serious PV. The considerable cost of the therapy limits its utilization in severe, recalcitrant or life threatening cases.

PU265
RESTRICTIVE GUIDELINES IN ALBUMIN PRESCRIPTION DOES NOT INCREASE MORTALITY AND REDUCES PHARMACEUTICAL EXPENSES
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Indications about the use of human albumin are still object of discussion. In fact, not only albumin is expensive, but it can also be dangerous when inappropriately administered. At the University Campus Bio-Medico, the clinical and economical impact of the adoption of the Italian Commissione Unica del Farmaco (CUF) guidelines for albumin utilization have been evaluated during the entire 2002. These guidelines, adopted during the year 2001, considered appropriate for albumin administration only the following two indications: a) evacuative paracentesis in cirrhotic patients, b) severe hydrosaline retention in ascitic cirrhosis or nefrotic syndrome, not responsive to adequate diuretic therapy. Based on that, we compared requests suitability, mortality’s incidence and albumin’s consumption during...
the entire 2002 and 1998 (year of the Cochrane Injuries Group Albumin Reviewers report about the correlation between mortality and albumin administration). Each albumin request was evaluated about suitability and conformity to guidelines in use during the respective year of request. For each period, the following parameters have been considered: total number of patients hospitalized in medical and surgical areas, total number of requests received, total number of requests accepted as appropriate, number of requests received/number of requests accepted, number of 20% albumin bottles administered per year, % of global mortality and global yearly weight of DRG. Results are summarized in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th># of hospitalized patients</th>
<th># of received requests</th>
<th># of requests accepted because appropriate</th>
<th># requests received/# requests accepted as appropriate</th>
<th># appropriate requests/# admitted patients (95% CI)</th>
<th># 20% Albumin bottles administered</th>
<th># 20% Albumin bottles/# of received requests</th>
<th>Global mortality rate</th>
<th>Global weight of DRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>4250</td>
<td>190</td>
<td>190</td>
<td>100%</td>
<td>4.4 (3.8-5.1)</td>
<td>1361</td>
<td>7.16</td>
<td>0.9</td>
<td>0.89</td>
</tr>
<tr>
<td>2002</td>
<td>2960</td>
<td>75</td>
<td>63</td>
<td>85%</td>
<td>2.1 (1.6-2.7)</td>
<td>351</td>
<td>4.68</td>
<td>0.7</td>
<td>1.18</td>
</tr>
</tbody>
</table>

In 1998, lacking the adoption of CUF guidelines, each albumin request received (n=190) has been accepted (100%), while in 2002, following the adoption of CUF guidelines, out of 75 albumin requests, 63 (85%) have been considered appropriate and approved. In 1998, albumin has been requested for 4.4% (95% CI: 3.8%-5.1%) of the hospitalized patients for a total of 1361 bottles and a medium number of 7.16 bottles for patient, while in 2002 albumin has been requested for 2.1% (95% CI: 1.6%-2.7%) of the hospitalized patients for a total of 351 bottles and a medium number of 4.68 bottles for patients. As a consequence, the adoption of CUF guidelines for the administration of albumin in hospitalized patients has produced a statistically high significant reduction (>50%) of the requests and a reduction of 35% in the amount of albumin bottles assigned to each patient, with a reduction in the pharmaceutical expense as for albumin purchase. Moreover, despite the reduction in albumin consumption mortality rate of patients did not increase in our hospital, even though the severity index of the hospitalized pathologies has been higher in 2002 than in 1998 (1.18 and 0.89 respectively). In conclusion, the adoption of CUF guidelines for albumin clinical use has permitted a more suitable drug utilization, a more correct management of the patients and an optimization of the resources, with a remarkable costs reduction that did not influence the mortality rate. Our aim is to extend the adoption of clinical and laboratory guidelines to other therapeutic and diagnostic procedures in order to improve the quality of assistance by controlling the costs.

PU266
ANEMIA IN CHILDREN WITH CANCER
Ruggiero A, Attinà G, Haber M, Lazzareschi I, Riccardi R
Divisione di Oncologia Pediatrica, Policlinico A.Gemelli Università Cattolica del Sacro Cuore, Roma, Italy

Anemia is a frequent and significant cause of morbidity in patients with cancer, that results in a decreased functional capacity and quality of life. The primary causes of anemia in children with cancer are different from those in adults; in adults decreased erythrocyte production is caused primarily by chemotherapy-induced aplasia of the erythroblastic cells lines and lowered production of endogenous erythropoietin, especially in platinum-based chemotherapy. It appears that in children with cancer, decreased erythrocyte production is related to bone marrow replacement by malignant cells and to direct effect of chemotherapy rather than by inadequate production of erythropoietin. However limited information is actually available about recombinant human erythropoietin as a treatment option in pediatric patients, so transfusions remains the standard therapy for treating cancer-associated anemia in children; because children are less sensitive to low levels of hemoglobin and because of risks inherent with transfusion, at the present time transfusions are limited to children with severe anemia. To better ascertain transfusion requirements in children with cancer and to identify the most important factors that increased transfusion requests, we conducted a retrospective chart survey of 124 children (62 CNS tumors, 38 solid tumors, 24 leukemias/lymphomas) treated from 1997 to 2001 at the Division of Pediatric Oncology, Catholic University of Rome (Table 1).

Table 1. Tumor type

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medulloblastoma</td>
<td>19</td>
</tr>
<tr>
<td>Low-grade glioma</td>
<td>23</td>
</tr>
<tr>
<td>High-grade glioma</td>
<td>4</td>
</tr>
<tr>
<td>Germ cell tumors</td>
<td>16</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>17</td>
</tr>
<tr>
<td>Non Hodgkin lymphoma</td>
<td>4</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>3</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>9</td>
</tr>
<tr>
<td>Rhabdomyosarcoma</td>
<td>9</td>
</tr>
<tr>
<td>Wilms tumor</td>
<td>4</td>
</tr>
<tr>
<td>Soft tissue and bone sarcoma</td>
<td>6</td>
</tr>
<tr>
<td>p-PNET</td>
<td>5</td>
</tr>
<tr>
<td>Others</td>
<td>5</td>
</tr>
</tbody>
</table>
Anemia was defined as mild (hemoglobin value > 10 g/dL), moderate (hemoglobin value 7-10 g/dL), and severe (hemoglobin value < 7 g/dL). Patients were divided according to chemotherapy regimen: mild, standard and intensive chemotherapy, which consisted of 2, 3 or >3 cytotoxic agents given at standard dosages respectively. More than 65% of children who received intensive chemotherapy requests transfusion; children who received standard and mild chemotherapy request less frequently transfusion (38% and 21% respectively) (Table 2). The prevalence of severe anemia and transfusion requirements increased if we considered patients receiving platinum-based chemotherapy (multivariate analysis is ongoing) (Table 3). This study suggests that the intensity of the treatment is one of the predominant factor in onset of anemia in cancer patients. Currently transfusion is the treatment most employed, but results of first few clinical trials of epoetin alfa in children with cancer-associated anemia are encouraging to offers a new and better therapeutic option.

Table 2: Chemotherapy Regimens and Frequency of Transfusions

<table>
<thead>
<tr>
<th>Chemotherapy regimens</th>
<th>Patients</th>
<th>Transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>31</td>
<td>5 (16%)</td>
</tr>
<tr>
<td>Intensive</td>
<td>25</td>
<td>4 (16%)</td>
</tr>
<tr>
<td>Standard</td>
<td>23</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Acenocumarol</td>
<td>16</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Phenprocoumon</td>
<td>19</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>Epoetin alfa</td>
<td>20</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

Table 3: Platinum administration and Frequency of Transfusions

<table>
<thead>
<tr>
<th>Chemotherapy regimens</th>
<th>Patients</th>
<th>Transfusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate (MTX)</td>
<td>31</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>25</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>23</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>16</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>19</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Methotrexate (MTX)</td>
<td>20</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

HEMOSTASIS, THROMBOSIS AND PLATELET DISEASES

Hemostasis and Thrombosis

COUMARIN-RESISTANCE: A CASE REPORT ILLUSTRATING A RARE CLINICAL CONCERN

Nicola P, Scaramucci L, Bongarzoni V, Morucci M, Montanaro M
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The inability of coumarins, widely used for oral anticoagulant therapy (OAT) for more ever extensive clinical indications, to elicit a therapeutic anticoagulant response in some patients may be due to several reasons. The different anticoagulant properties of these agents reflect their respective biological half-lives. Phenprocoumon, whose elimination half life is 144 hours, has a longer biological activity than racemic warfarin (half-life of 36 to 42 hours) and acenocumarol (half-life 10 h) but its effects are slower to reverse in case of over dosage or hemorrhagic complications. In Italy, the available anticoagulant agents are acenocumarol and warfarin, the latter being the most widely used, since it has a predictable onset and a longer effect, resulting in a more stable anticoagulation. The optimal warfarin dose required to achieve a therapeutic international normalized ratio (INR) varies greatly among individuals depending on a variety of factors, many of which are poorly understood. Different clearance and metabolism of warfarin linked to the demonstrated genetic polymorphism of cytochrome P 450 have been reported. We report on a patient, carrier of the Factor V Leiden thrombophilic anomaly, who became progressively resistant to acenocumarol and then warfarin. The replacement with phenprocoumon allowed to obtain adequate anticoagulation and to carry on the oral treatment. A 44-year-old male, presenting the FV Leiden thrombophilic anomaly, with a history of thromboembolic recurrences and a secondary pulmonary hypertension, who became progressively resistant to acenocumarol and then warfarin. The replacement with phenprocoumon allowed to obtain adequate anticoagulation and to carry on the oral treatment. A 44-year-old male, presenting the FV Leiden thrombophilic anomaly, with a history of thromboembolic recurrences and a suspected secondary pulmonary hypertension, who became progressively resistant to acenocumarol and then warfarin. The replacement with phenprocoumon allowed to obtain adequate anticoagulation and to carry on the oral treatment. A 44-year-old male, presenting the FV Leiden thrombophilic anomaly, with a history of thromboembolic recurrences and a suspected secondary pulmonary hypertension, who became progressively resistant to acenocumarol and then warfarin. The replacement with phenprocoumon allowed to obtain adequate anticoagulation and to carry on the oral treatment. A 44-year-old male, presenting the FV Leiden thrombophilic anomaly, with a history of thromboembolic recurrences and a suspected secondary pulmonary hypertension, who became progressively resistant to acenocumarol and then warfarin. The replacement with phenprocoumon allowed to obtain adequate anticoagulation and to carry on the oral treatment. A 44-year-old male, presenting the FV Leiden thrombophilic anomaly, with a history of thromboembolic recurrences and a suspected secondary pulmonary hypertension, who became progressively resistant to acenocumarol and then warfarin.

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heparin in spite of escalating doses of acenocumarol, successively replaced with warfarin, as also documented by his past medical record. The patient was not a vegetarian and did not have a history of malabsorption syndrome, liver or renal diseases. We prescribed gradually increasing warfarin until to 60 mg in three daily divided doses. The patient carried out the prescribed OAT with good compliance, failing to respond, resulting in the INR values never higher than 1.3 in four checks over two weeks. He received LMW heparin and warfarin was replaced with phenprocoumon. Patient was properly informed and gave his written consent. Although the recommended transition factor is 2.3 (1 mg of phenprocoumon is active as 2.3 mg of warfarin), we started the treatment with a loading daily dose of 9 mg (3 tablets), corresponding to about one third of reached maximal warfarin dose. The patient reached the therapeutic target within one week and carry on the treatment with phenprocoumon 7.5 mg mean daily dose. He was followed-up for 78 weeks, falling the INR in therapeutic range (2.5–3.5) in 25 (70%) and 8 (22%) out the 36 checks respectively. Over dose states were 3 (8%). The estimated time in range, calculated with the assumption of a linear increase or decrease between two consecutive INR determinations, was 70 (90%) out of 78 weeks. Patient experienced only one minor hemorrhagic complication (epistaxis) not associated with an over dosage (INR 3.1). Poor response to the coumarins can be managed increasing the doses, replacing the ineffective drug with another and, in the case of absolute resistance, with long-term administration of heparin. This report describes a case of acquired resistance to acenocumarol and warfarin. Patient initially responded to these drugs, presenting a delayed progressive lack of sensitivity to them. Our direct observation confirmed the resistance to warfarin, administered at very high doses, given that the patient was good compliant and all other causes of poor response has been excluded. The secondary resistance developed in long time may indicating a progressively acquired tissue resistance to acenocumarol and warfarin and a different variable affinity to their sensitive targets, the epoxide-reductate enzyme, or the progressively development of unknown metabolic changes (end-organ resistance). To date, the patient is on phenprocoumon and maintains good anticoagulation, although the follow-up is too short to exclude the development of resistance to this alternative agent too in the future. Our report on a poorly understood clinical concern has an anecdotal value. Larger longitudinal observations and pharmacogenomic studies aimed to improve our knowledge and the management of this rare phenomenon are warranted.

PU268
USE OF RITUXIMAB IN A CASE OF ACQUIRED HEMOPHILIA
Santoro R, Iannacarro PG, Papaleo G, Muleo G
Centro Emofilia, Servizio Emotassi e Trombosi, Azienda Ospedaliera “Pugliese-Ciaccio”, Catanzaro, Italy

Rituximab is a chimeric mouse/human anti-CD20 monoclonal antibody developed and licensed for the treatment of non-Hodgkin’s Lymphoma (NHL) originating from B cells. In the last years there have been an increasing number of reports of the use of this monoclonal antibody in disorders of humoral autoimmunity, like refractory autoimmune hemolytic anemia, chronic thrombocytopenic purpura, refractory autoimmune hemophilia. We report a case of a girl — 20-year-old — with acquired hemophilia resistant to conventional therapy. Our patient initially presented in 2001 with hemopterineum. She had a prolonged activated partial thromboplastin time factor VIII clotting (FVII:c) level of 2% of normal and FVIII:c inhibitor of $6$ Bethesda units. The patient started therapy with high doses of i.v. immunoglobulins and immunosuppression with prednisolone and, later, high doses of methilprednisolone, obtaining only a partial and transient rise of FVII:c. In subsequent months she became refractory to all forms of immunosuppression (cyclophosphamide, prednisolone, immunoglobulins, dexamethasone). In March 2003, after giving informed consent, she received rituximab at 375 mg/m$^2$ once weekly for 4 weeks. At this time she also received low doses of prednisone. Eight weeks after starting rituximab treatment, the FVII:c levels rose to 8% of normal and the inhibitory antibody was undetectable. The follow-up of the patient is still ongoing.

PU269
CLINICAL SIGNIFICANCE OF ANTI-COFACtor ANTibodies IN VENOUS AND ARTERIAL THROMBOEMBOLISM
Cattedra e Divisione di Ematologia, Ospedale S. Eugenio, Università Tor Vergata, Roma, Italy

Antiphospholipid antibodies make up a heterogeneous group of antibodies associated to thromboembolic events, recurrent abortions and low platelet levels. At the present time, it is diffused opinion that, certain coagulation proteins which bind to phospholipids are the true antigen target of antibodies. For this reason, along with cardiolipin (ACLA) and/or lupus coagulant (LAC), antibodies against protein-phospholipid complexes such as anti-β-2 glycoprotein 1, anti-prothrombin, anti-annexin V have acquired clinical evidence. We have studied
100 patients with previous thromboembolic events over a 12 months period; 19 were males and 81 were females with a median age of 36 (range: 21-66 years). The goal of this study was to verify if the level of anti-phospholipid antibodies and/or anti-cofactors was correlated to a higher thrombotic risk. Thrombotic events were present in 60% of cases in peripheral vessels (2/3 recurrent abortions and 1/3 TVP), in 20% of cases involving CNS and retina, and the other 20% ischemic heart disease and minor pathologies. Fifty-five percent of our patients presented familiar positivity for thrombotic events, and 15% circumstantial risk factors such as hypertension, lipidic dysfunction, neoplasies, pregnancy, estrogenic complications. Adverse interactions between warfarin prophylaxis, chemotherapy toxicity, or other parameters and INR elevation was observed between liver metastases, hepatocellular carcinoma, and hyperhomocysteinemia. The incidence of platelet hyper-aggregation was more evident, present in 43% of patients and hyperhomocysteinemia present in 40%; 2/3 of patients had developed an arterial thrombotic event, confirming the strict correlation of such parameters to athero-sclerotic pathology. The research of LAC, carried out according to the ISTH guidelines, turned out to be positive in 30% of our patients, 1/3 of which with autoimmune disease. Anti-cofactor antibodies, evaluated with an immuno-enzymatic method (Bouty), resulted high in 46 patients of which only 22 were LAC positive. In 72% of cases the anti-cofactor antibodies were associated to arterial thrombosis; 41% of our patients with elevated levels of anti-annexin V presented recurrent abortions even though they had normal LAC and ACLA levels. Although limited in time and evaluated in a restricted number of patients, our experience has revealed a good correlation between anti-cofactors and clinical manifestations. Particularly, the anti-annexin V antibodies resulted to be the only risk factor associated to recurrent abortion and therefore, assessable as an independent risk factor.

PU270
ADVERSE INTERACTIONS BETWEEN WARFARIN PROPHYLAXIS, 5-FUOROURACIL (5-FU) AND OXALIPLATIN IN PATIENTS WITH GASTROINTESTINAL TUMORS
Department of Medical Oncology and Hematology, *Department of Medical Radiology, Istituto Clinico Humanitas, Rozzano, Milan, Italy

Background. Prophylactic minidose warfarin in cancer patients with a central venous catheter (CVC) in place has an established role for the prevention of thrombotic complications. Adverse interactions between warfarin and 5-fluorouracil (5-FU) have been reported, but not so for warfarin and oxaliplatin (OHP). Aims. To assess if such an interaction exists, we performed a retrospective analysis on the incidence of International Normalized Ratio (INR) elevation in patients with advanced colorectal cancer receiving the FOLFOX regimen (5-FU 400 mg/m² as a 2-hour i.v. infusion on days 1 and 2; 5-FU 600 mg/m² as a 22-hour i.v. c.i. on day 1 and 2; folinic acid 100 mg/m² as a 2-hour i.v. infusion on days 1 and 2 plus OHP 85 mg/m² as a 2-hour i.v. infusion on day 1) with concomitant prophylactic minidose warfarin. Patients and Methods. Between 7/2000 and 2/2003, 68 evaluable patients (M/F:41/27, median age: 62 years, liver metastases: 48) were evaluated for INR values as well as bleeding. All patients were affected by metastatic colon cancer. The plan was to give chemotherapy until evidence of progressive disease or unacceptable toxicity. Warfarin (1 mg/day) was started the day of CVC insertion. INR was measured before CVC insertion (baseline) and at least four times, afterwards. Results. The normal value of INR was 0.90 to 1.18 with an INR more than 1.5 being regarded as significantly elevated. Among the patients, the median INR value at baseline was 1.09. Altogether, 352 INR determinations were performed. The median number of INR determinations per patient was 5 (range 4-9). INR elevation was observed in 27/68 patients (40%) ranging from 1.55 to 9.4 (mean 3.14). Of these, 4 patients had an INR > 3.0 < 6.0 and 4 had an INR > 6.0. The median time to INR elevation was 69 days (range 14-160 days) from the start of chemotherapy. Two patients developed hematuria requiring hospitalization, with INR levels at the time of 7.0 and 7.38, respectively. Minor bleedings were observed in 4 patients with normal INR level. We did not observe abnormal hepatic parameters during chemotherapy and no relationship was observed between liver metastases, chemotherapy toxicity, or other parameters and INR elevation. Conclusions. Clinicians should be aware of this interaction and should regularly monitor the INR level in patients receiving FOLFOX chemotherapy and minidose of warfarin.

PU271
SPLENIC ARTERY EMBOLIZATION: IN WHICH HEMATOLOGICAL PATIENTS?
Filardi N, Molfese V,* Pizzuti M, Attolico I, Vertone D, Ricciuti F
U.O. di Ematologia and *Radiologia Ospedale San Carlo, Potenza, Italy

Background. The indications for splenectomy include: abdominal traumas, hypersplenism with peripheral cytopenia (hereditary spherocytosis - HS-, thalassemia, idiopathic thrombocytopenic purpura -ITP-, acquired hemolytic anemia) and splenic localization of hematological disorders (lymphomas, chronic myeloproliferative disorders). Besides conventional laparotomic...
splenectomy, we make use of other techniques such as total laparoscopic splenectomy, with or without previous splenic artery embolization (SAE) and partial splenectomy (splenic artery embolization and splenic irradiation). Aim. We evaluated efficacy and safety of splenic artery embolization in patients with hematological disorders who were not eligible for laparotomic splenectomy because of age, patients’ refusal or poor clinical conditions. Methods. SAE was performed in 5 patients, 2 male and 3 female, with median age of 74 years (range 60–89), because of ITP in 3 cases, HS in 1 case and idiopathic myelofibrosis (IM) in 1 case. All the patients underwent anti pneumococcal, anti meningococcal, anti H. influenzae vaccination 14 days before the procedure. After right femoral artery access, catheterization of coeliac axis and then of splenic artery was performed with idrophilic material (Terumo). Embolization was obtained through non magnetic spiral (Cook 8mm × 5 cm) and grated sponges of Spongostan (Zimospuma). Results: Good responses were obtained in the patient with HS and in two of the three cases with ITP. One patient with ITP had only a partial recovery in platelets count, maybe because Spongostan had not been used. Side effects were transient and acceptable in all patients but the one with IM, who died of shock probably due to massive necrosis of the large spleen, three days after the embolization. Results and characteristics of the patients are summarized in the table.

Conclusions: SAE, after vaccinations, is a safe and effective alternative in patients with hematological disorders who are not eligible for laparotomic or laparoscopic splenectomy because of age, refusal or poor clinical conditions. Our little experience suggests to avoid this procedure in patients with massive splenomegaly.

PU272
COAGULATION DISORDERS IN MIGRAINES WITH AURA: A REPORT OF 42 CASES
Dipartimento Ematologia, S. Prevenzione e Cura
Sindromi Emorragiche e Trombotiche,
+Servizio Neurofisiopatologia, Ospedale Civile dello
“Spirito Santo”, Pescara, Italy

Migraines have been shown to be an independent risk factor for ischemic stroke in men and in pre-menopausal woman. Patients with migraines without aura can suffer from cerebral ischemic events, but the association between migraine with aura (MA) and cerebral infarction is more prominent. The nature of this connection remains essentially unknown and possible mechanisms predisposing young migraineurs to ischemic stroke may be vascular, neuronal or related to coagulation abnormalities. In particular, the presence of antiphospholipid antibodies, has been reported in some studies. Recently, newly discovered inherited clotting defects which might play a role in hypercoagulable states (resistance to activated protein C due to Factor V mutation, factor II 20210 mutation, antithrombin, protein C, protein S deficiencies, elevated factor VIII levels and hyperhomocysteinemia), have been described. There are also some intriguing observations regarding essential thrombocytopenia (ET) that suggest an increased frequency of migrainous auras. In view of these considerations we decided to investigate the presence of coagulation abnormalities in migraine with aura patients. From June 2002 to May 2003 we recruited a consecutive series of MA patients diagnosed at the Headache Center of our Hospital. The diagnosis of MA was based according to International Headache Society (HIS) classification 1988. In parallel, an age and sex matched control group of 42 healthy subjects was recruited. Characteristics and results are shown in Tables 1 and 2.

Table 1. Characteristics.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age (mean±SD)</th>
<th>Sex F/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine with aura</td>
<td>42</td>
<td>35±11</td>
<td>34/8</td>
</tr>
<tr>
<td>Controls</td>
<td>42</td>
<td>39±11</td>
<td>36/6</td>
</tr>
</tbody>
</table>
Table 2. Coagulation abnormalities in patients with migraine with aura and the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Factor V Leiden Mutation</th>
<th>FII up levels to 150 UdL⁻¹ fasting</th>
<th>FVIII:C</th>
<th>Hyper Homocysteinemia positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraines with aura</td>
<td>42</td>
<td>n 3 (7.1%)</td>
<td>n 1 (2.3%)</td>
<td>n 8 (19%)</td>
<td>n 8 (19%)</td>
</tr>
<tr>
<td>Control</td>
<td>42</td>
<td>n 1 (2.3%)</td>
<td>n 1 (2.3%)</td>
<td>n 4 (9.5%)</td>
<td>n 3 (7.1%)</td>
</tr>
<tr>
<td>p-value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

Three cases of ET were also found in the migraine with aura group, but statistical analysis is not possible. Some authors consider migraines with aura a blood disorder due to increased platelet activation, detected both during attacks and in the headache-free period. Our observation suggests that migraines with aura should not be a single entity but epiphenomenon of coagulation alterations linked to a thrombotic tendency. The results of this study also indicate that aCL activity is more present in young adults who suffer from MA than in control group. A study on ET in MA patients with high platelets count should be encouraged considering that the annual incidence rate of ET in the general population is 1.4/100,000 but our study found this disease in 3 out of 42 MA patients. Further studies on larger groups are thus urgently needed to establish the role of hemostasis in MA.

**Table 1. Characteristics of the subjects included in the study.**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Age (26-57)</th>
<th>Months the use of OCs (3-112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>34</td>
<td>12</td>
</tr>
</tbody>
</table>

**Table 2. Coagulation abnormalities for cerebral vein thrombosis in oral contraceptive users.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>n</th>
<th>FII Mutation and Hyperhomocysteinemia fasting</th>
<th>FII</th>
<th>FVIII:C levels up to 150 UdL⁻¹ fasting</th>
<th>Hyperhomocysteinemia fasting</th>
<th>aCL positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
</tr>
</tbody>
</table>
The presence of other causes of thrombophilia (deficiency of antithrombin III, protein C, or protein S) was excluded. Conclusions: Oral contraceptives are associated with an increase of CVT risk by a factor of 20. The combined effect of the genetic or acquired risk factors in OC users greatly increases the risk of cerebral-vein thrombosis and the most frequent hereditary thrombophilic condition is the G20210A mutation in the prothrombin gene. As shown in our series of patients with cerebral-vein thrombosis, a hypercoagulable state in oral contraceptives users is strongly associated with cerebral-vein thrombosis.

PU274
THE EFFECTIVENESS OF RECOMBINANT ACTIVATED FACTOR VII THERAPY IN A 92-YEAR-OLD MALE WITH ACQUIRED HEMOPHILIA
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Acquired hemophilia is a rare bleeding disorder caused by the development of specific auto-antibodies which are capable of inhibiting the action of naturally occurring factor VIII (FVIII). The clinical manifestation is a severe and often life-threatening bleeding tendency with an overall mortality of 15-22%. Therapeutic decisions should be based on the severity of bleeding and laboratory characterization of the anti-FVIII antibody. The bleeding can be controlled using concentrates of human or porcine FVIII, as well as recombinant VIIa (rFVIIa; NovoSeven(r) - Novo Nordisk). We report the case of a patient 92-years-old male admitted to our hospital due to a post-traumatic massive hematoma involving the left shoulder, dyspnea and fatigue. Laboratory evaluations were performed and revealed normocytic, normochromic anemia (hemoglobin 7.8 g dL-1), leukocytosis and neutrophilia (WBC 15.6x10^9/L; neutrophil count 13.6x10^9/L); prolonged Partial Thromboplastin Time (aPTT 45", ratio 1.32) and normal PT (PT ratio 1.07). The mixing test with normal plasma showed a prolonged aPTT. Decreased coagulant activity of FVIII (FVIII:C 14%) was detected, with a title of inhibitor against factor VIII of 1.5 U/mL; the von Willebrand factor activity was increased to 260%. All conditions known to be associated with secondary acquired hemophilia were excluded. The patient was immediately treated with 2 units of packed red blood cells and because of the low inhibitor titre, therapy with Human FVIII was started with 4000 U of FVIII, for neutralization, followed by 3000 U every 8 h for substitution. Despite 3 consecutive days of substitutive therapy, the patient showed an enlargement of left shoulder hematoma, a mild rise of hemoglobin levels (Hb 8.3 g/dL). Due the failure of substitutive therapy, human FVIII infusions were stopped and, in attempt to control the bleeding, 90 mg kg-1 of rFVIIa were given intravenously, and 3 hours later repeated, without side effects or evidence of thrombotic events. No antifibrinolytic drugs were added. Bleeding immediately stopped and the patient had no further blood loss. On day 8 a second distinct bleeding episode occurred with a spontaneous hematoma on the right arm and hemithorax. Haemoglobin fell from 8.2 to 6.3 g dL-1 in 24 hours. This episode was also treated with two rFVIIa infusions and anemia was corrected with two units of packed red blood cells. Bleeding stopped and the patient remained asymptomatic, without clinical re-emergence of bleeding. On the 11th day after admission, the patient developed acute respiratory failure due to a pneumonia and deceased. Acquired hemophilia remains a life-threatening condition associated with a high mortality, mainly in elderly patients. Various treatment modalities are available to halt the bleeding diathesis and rVIIa has showed to be an effective salvage therapy for bleeding in patients with acquired hemophilia who failed to respond to blood-product therapy. Treatment with rFVIIa has been associated with a low incidence of thrombotic complications, possibly due to low levels of free thrombin generation, but thrombogenicity remains a theoretical risk when using rFVIIa, mainly in elderly patients, who frequently have additional thrombotic risk factors. Our case confirms previous data reporting the complexity and severity of this disorder and showing that rFVIIa is an effective and safe agent for achieving hemostasis also in elderly patients with an inhibitor to FVIII.

PU275
THROMBOTIC THROMBOCYTOPENIC PURPURA AS METASTATIC LUNG CANCER FIRST SIGN
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On July 2002, a previously healthy 66 year-old-woman, M M, was admitted to our medical unit for asthenia, fever, weight loss, anemia and trombocytopenia. Blood tests were as follows: hemoglobin 8.4 g/dL, red blood cells 2.430.000/µL, platelets 43000/µL, reticulocyte count 11%, total bilirubin 2.0 µg/dL, indirect bilirubin 1.5 µg/dL, LDH 1212 U/L, haptoglobin 29 µg/dL, D-dimer 825 ng/dL, coagulation tests, fibrinogen and creatinine were normal. A diagnosis of thrombotic thrombocytopenic purpura (TTP) was made, supported by bone marrow and blood smear findings. Treatment was promptly instituted with methylprednisolone (starting dose 1 g/kg) and plasma exchange (average volume exchange ranged ranged 20-40 mL/kg
Glanzmann’s thrombasthenia (GT) is an autosomal recessively inherited bleeding disorder, characterized by the complete absence of platelet aggregation. Quantitative or qualitative abnormalities of the glycoprotein complex IIb-IIIa are responsible for this disorder. Patients affected by GT show a severe and life-long bleeding tendency and among such patients just one case of deep vein thrombosis, treated with heparin, has been described. We report the case of a GT patient (diagnosis made at age of 22 years) who presented a deep vein thrombosis in the posterior tibial vein of right leg, at the age of 38 years, confirmed by color-doppler ultrasonography. This event was spontaneous and it happened when the patient was completely healthy. The patient has always shown a moderate/severe bleeding tendency and the more frequent bleeding events since childhood have been epistaxis and, later, gum bleeding. The molecular variant of GT of our patient is characterized by a homozygous deletion of Cys49 in the b3 exon 3 (224Gdel). Heparin treatment, at therapeutic dosages, has begun and then substituted by oral warfarin (dicumarol). Therapeutic target of INR was 2 and a very close clinical and laboratory follow-up was instituted. Thrombophilic screening showed the absence of antiphospholipid antibodies and normality of Antithrombin, Protein C and Protein S levels. No resistance to activated protein C (APC) was present. Molecular biology tests showed that the patient was heterozygous for PAI-1 polymorphism (4G/5G), and heterozygous for MTHFR C677T and MTHFR A1298C. Resistance to activated protein C (APC) was present. Molecular biology tests showed that the patient was heterozygous for PAI-1 polymorphism (4G/5G), and heterozygous for MTHFR C677T and MTHFR A1298C.

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PU277
IDIOPATHIC THROMBOCYTOPENIC PURPURA IN PREGNANCY: MANAGEMENT AND TREATMENT
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As platelets play an important role in primary and secondary hemostasis, any decrease in their count in peripheral blood causes justifiable concern. This is particularly the case during pregnancy and decreased platelet count can be due to immune microangiopathic and consumptive mechanism. Idiopathic thrombocytopenic purpura (ITP) is occasionally manifested during pregnancy by routine prenatal screening for blood cell counts infact occurs more commonly in young women during the reproductive years. It is an autoimmune disorder in which antiplatelet autoantibodies bind to the antigens on platelet surface resulting in their destruction. ITP can potentially cause serious hemorrhage both in mother and fetus, accurate assessments as well as appropriate clinical managements are necessary for successful delivery. The main options of treatment of ITP are corticosteroids, intravenous immunoglobulin (IVIG) and splenectomy. We observed five pregnant women with idiopathic thrombocytopenic purpura in collaboration with our Obstetric and Gynecologist division. All the mothers received corticosteroid therapy during the last month of gestation, two unsponsored patients received high doses immunoglobulin therapy before delivery. At the time of delivery four mothers had normal platelet counts and one had low platelet count. Four deliveries were by vaginal route and one were by cesarean section. All infants were born with normal platelet counts. No infants showed any clinical signs of hemorrhage and there were no neonatal complications. In conclusion we think that thrombocytopenia may be a physiological phenomenon of pregnancy, or the end result of an autoimmune disorder. ITP requires the management of two patients, the mother and her baby and requires the close collaboration of a group composed by hematologist, obstetrician, anesthesiologist and neonatologist.

PU278
ANTIPHOSPHOLIPID SYNDROME AND PREGNANCY: CASES REPORT
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Antiphospholipid syndrome (APS), a condition characterized by one or more thrombotic or pregnancy-related clinical features in association with medium to high levels of antiphospholipid antibodies, has emerged as an important diagnostic consideration in several medical fields. The antiphospholipid syndrome is defined by the occurrence of venous and arterial thromboses, often multiple, and pregnancy morbidity (mainly, recurrent fetal losses and premature births), frequently accompanied by a moderate thrombocytopenia, in the presence of antiphospholipid antibodies (aPL) namely lupus anticoagulant (LA), anticardiolipin antibodies (aCL), or both and is one of the few treatable causes of pregnancy loss and successful pregnancy of 70% or more can be achieved with appropriate treatment. Antiphospholipid antibodies are a heterogeneous family of immunoglobulins and despite their name do not recognize phospholipids, but plasma proteins bound to suitable anionic (not necessarily phospholipid) surfaces. The relationship between lupus anticoagulants and anticardiolipin antibodies and thrombosis has been amply investigated. A high level of antiphospholipid antibodies and anticardiolipin antibodies was detected. We report the cases of three patients with a history of recurring pregnancy loss and positive preconception screening for antiphospholipid syndrome. After confirmation of a viable pregnancy all subjects began taking 4000 UI of Low molecular weight heparin (LMWH) once daily subcutaneously until delivery and it was stopped twelve hours before and restarted twelve hours after but it was stopped two weeks after discharged. None had hemorrhagic or thrombotic complications during pregnancy and delivery and all the new-borns had good health. We think that, in females with history of recurring pregnancy loss, a good diagnostics that include finding antiphospholipid antibodies, anticardiolipin antibodies and lupus anticoagulant and an appropriate management strategies using low molecular weight heparin a greater that 70% live birth rate may be achieved.

PU279
IDIOPATHIC ACQUIRED HEMOPHILIA IN THE ELDERLY: A CASE REPORT
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Summary: Acquired hemophilia (AH) is a rare coagulopathy occurring most frequently in elderly patients, caused by formation of autoantibodies directed against factor VIII (FVIII). Although autoimmune diseases, hematologic malignancies, solid tumors can be recognized, in almost half of the cases autoantibodies develop spontaneously without an underlying medical condition. Serious and often devastating life-threatening hemorrhagic complications, associated with consider-
able morbidity and mortality, characterize the disease. However a complete remission is possible, if the treatment results in recovery of factor VIII activity. Patients and methods: A 81-year-old non-hemophiliac woman was admitted to our Unit because of acute severe anemia (hemoglobin 8.3 g/dL) associated with spreaded hematoma in soft tissues of her right upper limb. Coagulation studies revealed an increasing marked prolongation of activated partial thromboplastin time, (88 to 102 seconds, control: 21-39), a diminished activity of factor VIII (2.4%, control 50-150%) in spite of intensive transfusional support. Neither tumoral markers, specific autoantibodies, antiglobulin test positivity, rheumatoid factor, lupus anticoagulant were found nor previous meaningful clinical conditions. A significant increase of factor VIII inhibitor titer (9.36 Bethesda unit/mL) was finally detected. High-dose steroids (6 metilprednisolone 1 mg/kg/day i.v.) were administered. One week later (aPTT = 44.5 seconds) an increasing pain in the left inguinal region revealed a massive abdominal retroperitoneal hemorrhage (diameter 92×71 millimeters). The hemorrhage quickly spread over the soft tissues fully affecting the back, the left side and the left lower limb. Severe anemia rapidly occurred (hemoglobin from 10 to 7.9 g/dL). Results: Recombinant factor VII, erytrocytes transfusions and steroids were inadequate to control bleeding. High-dose prothrombin complex concentrates (FEIBA) in association with antifibrinolytic therapy successfully stopped expansion of the hematoma in soft tissues of her right upper limb. Coagulation measurements at the cessation of bleedings and prevention of their relapse. Bleeding may be controlled by factor VIIa recombinant or prothrombin complex concentrates. Corticosteroids are useful, but can be highly toxic, especially in the elderly. A prolonged surveillance of each case of idiopathic AH is mandatory because its long-term outcome is still largely unknown.

Funding: Supported in part by Pesaro AIL-Onlus.
Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by recurrent thromboses, spontaneous abortions and thrombocytopenia. It can be primary (idiopathic) or secondary, i.e. associated with autoimmune diseases or neoplasms mostly of lymphoid origin. Rituximab is a monoclonal humanized anti-CD20 antibody capable of producing a prolonged immunosuppression through B-cell depletion. The use of chemotherapy in APS associated with lymphomas was reported as ineffective in reverting titers of autoantibodies, outside the contest of bone-marrow transplantation. The use of rituximab could in some recent reports cancel the titre of antiphospholipid antibodies, and stop cardiovascular events in symptomatic patients. We report on 2 cases of APS and lymphoma. Case 1: a 53-years old man, with a marginal zone NHL of the spleen (SMZL) diagnosed at bone marrow biopsy, presenting with splenomegaly, symptomatic deep bilateral venous thrombosis of the femoral veins and pulmonary embolia. HbsAg positivity. LAC and APA positivity at high titre (>100 UI/mL). We started treatment with ev eparin and chemotherapy (CHOP); a severe progression in venous involvement during eparin infusion was registered, until a catastrophic clinical picture of venous thrombosis involving the four limbs. After the first cycle of therapy we obtained a reduction of splenic enlargement without control over the progressive venous thrombosis. At the second cycle of chemotherapy we added Rituximab (375 mg/m²) to CHOP. We obtained a progressive reduction of splenomegaly and improvement of control over clinically evident DVP, with the disappearance of both APA and LAC titers after four cycles of chemotherapy. The patient underwent splenectomy for the persistence of splenic enlargement with echographic evidences of splenic lesions; histology was negative for NHL and the patient is now in complete remission of the lymphoma. Anticoagulant therapy was stopped. Case 2: a 42-years old man with a previous arterial thromboembolism of the lower limb and amputation of the right foot. High titers of APA and LAC had been found 5 years before the diagnosis of NHL during routine examination before surgical mitral valvular substitution. After this operation the patient was treated with continuous oral anticoagulant treatment. The lymphoma (DLBCL) presented with lymphadenopathy, systemic symptoms, splenic enlargement with a large hypodense lesion. High
titers of APA and LAC were confirmed. We started treatment with CHOP and Rituximab (375 mg/m²), obtaining a partial response. We decided to continue with VACOP-B regimen adding Rituximab (4 cycles along 8 cycles of VACOP-B). At the end of treatment we documented a complete remission of the lymphoma and the APA titer was negative. Our observations support the possibility to induce prolonged immunosuppression with disappearance of antiphospholipid titers by the use of chemotherapy and rituximab in the treatment of APS associated with lymphoid neoplasms; in case 1 we could manage this way to stop a highly symptomatic, catastrophic deep venous thrombosis that was progressive despite the use of endovenous eparin in continuous infusion.

PU283
THROMBOPHILIC SCREENING IN PATIENTS WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is associated with thromboembolic events (VTE). A higher incidence of VTE is observed when the disease is treated with first-line thalidomide. It should be assessed whether or not personal baseline coagulation alterations may interact with the type of disease and thalidomide treatment thus contributing to such increased incidence of VTE complications. In the present study we investigated 92 patients with MM, M/F 46/46, median age 58 years (range 35-68), by screening them with a complete thrombophilic study (prothrombin time, activated partial prothrombin time, hepatoquick, antithrombin III (ATIII), protein C and S activities, activated protein C (APC) resistance, Factor V Leiden and G20210 prothrombin mutation) to assess whether or not a baseline condition of hypercoagulability was present. As shown in the table the following alterations were detected at baseline: low AT III in 2 pts, low protein C and protein S activity in 3 and 2 pts, respectively; these alterations were associated with a low hepatoquick test and were most likely due to a reduction of liver function. Two patients (2.2%) had a factor V mutation (R506Q) and 4 patients (4.4%) had a G20210A prothrombin mutation; the prevalence of these congenital alterations is not different from that expected in an unselected population. Twelve patients showed an APC resistance in the absence of factor V Leiden mutation (according to Bertina; cut-off 0.8 normalized ratio). Six of these patients could be tested after a period of 4 month treatment with thalidomide and dexamethasone: 5 of them had a normal APC resistance and so we can exclude the presence of some factor V polymorphisms other than factor V Leiden. One patient, carrier of the G20210A prothrombin mutation, showed a persistent APC resistance. We conclude that pts with multiple Myeloma seem not to have an higher incidence of congenital thrombophilic defects; the relatively frequent alterations of physiologic blood coagulation inhibitors recorded at baseline contribute to a condition of acquired hypercoagulability and are likely the results of liver disfunction or other alterations associated with the baseline disease.

PU284
ACQUIRED HEMOPHILIA AFTER AUTOLOGOUS TRANSPLANT (AUBMT) FOR MULTIPLE MYELOMA

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A 58 y-old male affected by MM IgGk IIIA (MC 7.4 g/dL; BM plasma cells:40%; Hb 11.9 g/dL; X-rays: large peri-acetalobular osteolysis; Tc99m-sestaMIBI: diffuse marrow uptake with focal pelvis uptake) was treated with chemotherapy and a double aUBMT (last procedure in April 2001) attaining CR (absence of MC by immunofixation, normal basal work-up, normal Tc99m-sestaMIBI scan, absence of symptoms). In March ’03, still in CR and in maintenance treatment with α-interferon (according to the treatment protocol), large spontaneous hematoma of the limbs appeared, with evidence of prolonged APTT (73”). Interferon treatment was interrupted, but coagulation parameter did not change and severe hematuria developed soon after, which resolved with hydration and antibiotics. Low level of factor VIII activity (4%) and antagonist VIII inhibitory activity (3.4 U.B.) were documented, leading to recurrent non severe bleeding episodes in subcutaneous tissue or in muscles. Acquired hemophilia is a rare bleeding disorder caused by autoantibodies inhibiting the function of
coagulation factors, mainly factor VIII. Autoimmune disease are the conditions most frequently associated, followed by the peri partum period, by malignancy and by drug reactions. At our knowledge, no other cases of development of anti factor VIII autoantibodies have been reported after auBMT. In our case, identification of Ig isotype, possible relations to the underlying immunoproliferative disease or to some derangement of immune function reconstitution after auBMT and treatment plan will be addressed in the near future.

Platelet Diseases

The effects of continuous intravenous infusion of dipyridamole as adjunctive therapy in the treatment of thrombotic thrombocytopenic purpura
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Thrombotic thrombocytopenic purpura (TTP) is a rare hematologic disorder characterized by thrombocytopenia, microangiopathic hemolytic anemia, and ischemic events often occurring in the nervous system and kidneys. The advent of plasma exchange has dramatically improved the prognosis of disease, since it is associated with a survival rate of 78% at 1 month. However, mortality rates remain significant. A variety of adjunctive treatments have been used in association with plasma exchange and antiplatelet drugs have been widely used. In this pilot study we investigated the effects of an adjunctive therapy consisting of the continuous, intravenous infusion of dipyridamole, a modality of administration that has not been previously tested in this setting. Sixteen untreated TTP patients, (12 women, 4 men) with a mean age of 39 years, received daily plasma exchange together with intravenous methylprednisolone (1-2 mg/kg/twice daily) and a continuous infusion of dipyridamole (100 mg/24 hours/day). A complete response was defined as an improvement in the platelet count to more than 150×10⁹/L for two consecutive days and no neurologic deterioration. When complete remission was achieved, plasma exchange and dipyridamole were stopped and methylprednisolone was gradually tapered. The overall response rate was 87.5%. Platelet counts increased to above 50×10⁹/L within 2 to 9 days and a normal platelet count was attained within 4 to 29 days in fourteen patients. One patient failed to respond to the combination therapy but attained a consistent remission after autologous stem cells transplant. One patient was refractory to the combination therapy and died, after an initial but unsustained response. All fifteen patients are currently in remission 1 to 5 years after diagnosis. This study suggests that the addition of a continuous infusion of dipyridamole to plasma exchange and steroids in the management of acute TTP is safe and well-tolerated. The results of this pilot study demonstrate that the addition of dipyridamole did not reduce the remission rate usually obtained with conventional treatment, but the potential clinical value of this approach will have to be validated in the context of a proper randomised trial.
but may be frequent. which is a phenomenon that has still to be determined, between the erythroid and megakaryocyte cell lines, explained as a consequence of competition for iron. 

Discussion and conclusions: thrombocytopenia during iron replacement therapy could be explained as a consequence of competition for iron between the erythroid and megakaryocyte cell lines, which is a phenomenon that has still to be determined, but may be frequent.

PU287

IMMUNE THROMBOCYTOPENIA INDUCED BY FLUDARABINE IN A MARGINAL ZONE LYMPHOMA PATIENT


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Fludarabine, a purine nucleoside analog, is an effective agent in the treatment of a number of lymphoproliferative malignancies. This agent is used in several hematologic diseases such as chronic lymphocytic leukemia (CLL), low-grade non Hodgkin’s lymphomas, Waldenstrom’s macroglobulinemia, acute myeloid leukemia and as a conditioning agent in non myeloablative stem cell transplants. The most common fludarabine toxicities are myelosuppression and immunodeficiency with consequent development of opportunistic infections. Fludarabine is also associated with the development of autoimmune hemolytic anemia and, rarely, with pure red-cell aplasia and immune thrombocytopenia. Here we report a severe autoimmune thrombocytopenia arising after four courses of fludarabine in a patient affected of marginal zone B-cell lymphoma. A 71 year old man was diagnosed in August 2000 with marginal zone lymphoma. He was initially treated with a radio and chemotherapy including four courses of CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisone) and involved field radiotherapy obtaining a complete response. In July 2002 he relapsed developing retroperitoneal lymphadenopathy and parotid gland involvement. The histological examination confirmed the presence of a low-grade non Hodgkin’s lymphoma of the marginal zone B-cell histological subtype. No bone marrow lymphoma involvement was present. A Fludarabine (25 mg/m² for 3 days) associated with cyclophosphamide (300 mg/m² for 3 days) regimen was started for a total of four courses. The patient obtained a good partial response. Before fludarabine treatment, the platelet count was normal (185.000/m³). After the fourth course of therapy the platelet count decreased progressively up to 17.000/m³ on the 38th day from the last fludarabine somministration. Bone marrow examination showed normal myelopoiesis and in particular adequate megakaryocytopenia. Antiplatelet antibodies (PAI) were found positive (IgG). Standard treatment with oral prednisona at the dose of 1 mg/kg/day and intravenous immunoglobulin (Igv) 0.4 g/kg/day for 5 days was begun. Ten days later, because no significant platelet response was observed, high dose dexamethasone at the dose of 30 mg daily for 4 days every two week was started with only a partial response. ITP arising after fludarabine therapy is considered a rare event and, at the best of our knowledge, it has been reported only in few CLL cases. All these patients developed an acquired severe thrombocytopenia obtaining variable responses to standard interventions. Here we first describe at case of thrombocytopenia arising in a B-cell low-grade non Hodgkin’s lymphoma different from CLL, in which no previous bone marrow involvement nor lymphoma associated autoimmune disorders were present, that was strictly related to the fludarabine treatment.

PU288

IDIOPATHIC IMMUNE THROMBOCYTOPENIC PURPURA IN ADULT PATIENTS: A STUDY OF 200 PATIENTS AFFERED IN A SINGLE ISTITUTION

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Background. Adult ITP is one of the most common causes of thrombocytopenia and the incidence has not been determined precisely; the first line therapy (steroid ± immunoglobulins) induce complete response (CR) or partial response (PR) about 65-75% of cases and the splenectomy is efficacy in 70-80% of patients with resistance or relapsed disease. Aim: Evaluate clinical characteristics at the presentation, the results with therapy and outcome in group of patients with affered in a single istitution. Results. From May 1, 1998 to April 30, 2003 200 adult patients affected by ITP were diagnosed in our center; all patients were negative for HCV/HIV infection or LAC/aCAs or presence autoimmune disease; only 50 patients were positive for platelet’s autoantibodies; the median age was 42 years (range 18-86); 143 were female and 57 male (ratio 2.5); the median count of platelets at diagnosis was 35 x10⁹/L (range 2-110x10⁹/L); at diagnosis 60 patients showed minor bleeding events and 10 patients major bleeding events (2 patients intracranial haemorrhage and 8 patients gastrointestinal); 103 patients were treated (70 steroid and 33 with steroid plus immunoglobulins); 66 patients achieved a a major response (56 CR and 10 PR); in 27/37 of patients (were not response) splenectomy was performed and 90% achieved a major response; in 10/37 (were not response)a second lined therapy (3 cyclosporine, 5 cyclophosphamide, 2 azatioprine) was administred with
only 30% of response; with a median follow up of 46 months, 10 patients relapsed and only 1 patients died for major bleeding event. 9/10 patients who relapsed were re-treated: 4 patients only steroid (3/4 CR e 1 minor response), 4 cyclosporine (2 CR, 2 not responded), 1 with rituximab (1 CR). Conclusions: in our experience the adult ITP is relative rare disease; the first line therapy (steroid +/- immunoglobulin) is effective and in second line splenectomy is efficacy.

**PU289**

**TREATMENT OF TWO PATIENTS WITH ACQUIRED FACTOR VIII INHIBITOR USING CYCLOPHOSPHAMIDE AND CORTICOSTEROIDS**

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Blood coagulation is one of the major physiological mechanisms involved in the arrest of bleeding at the site of injury and hence, hemostasis. Patients with congenital or acquired hemophilia have defective coagulation processes ad are at risk of serious bleeding complications. Acquired hemophilia is a rare disorder due to spontaneous development of antibodies directed against the factor VIII molecule in patients with previously normal levels of factor VIII. Antibodies against factor VIII can cause life-threatening bleeding especially of soft or subcutaneous tissue or in muscle requiring costly factor replacement and prolonged immunosuppression. In 73% of the patients, the FVIII inhibitor appear either during pregnancy or in the postpartum period and is associated with considerable morbidity as well as mortality especially in the elderly patients. The aPTT prolongation, the reduction of the factor activity and the inhibitor titre are of diagnostic importance. We observed two cases of acquired hemophilia admitted to our hematology section due to mild bleeding of soft or subcutaneous tissue or in muscle. One of them was in postpartum period, other patient was elderly. The coagulation assay showed aPTT prolongation and FVIII procoagulant activity was low with a Bethesda assay confirming the presence of anti human FVIII antibody. Diagnosis of acquired hemophilia was confirmed and they were treated with corticosteroid therapy and cyclophosphamide. The patients had a good resolution of the antibody and of mild bleeding without any substitutive therapy. We think that a correct evaluation of coagulation screening test, in particular aPTT, is essential, moreover for treatment of acquired hemophilia immunosuppressive agents combined with corticosteroids and occasionally with aFVII administration and/or immunoadsorption may lead to long-term inhibitor eradication reducing bleeding.

**PU290**

**THALIDOMIDE THERAPY IN REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA ASSOCIATED WITH MULTIPLE MYELOMA**

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Immune thrombocytopenic purpura (ITP) is often seen in patients with lymphoproliferative disorders, few cases have been described in multiple myeloma (MM) patients. A variety of agents such as Immunoglobulins, Danazol, Azathioprine have been used in refractory ITP, none of these agents has antimyeloma activity. Thalidomide is effective in MM patients and in several autoimmune diseases. With this rationale we tried this approach in one MM patient with associated ITP: a complete response was achieved and maintained for 36 months. On October 1994 a diagnosis of IgGk MM was established in a 43-year-old man. In December 1994 disease recurrence occurred and high dose chemotherapy with peripheral blood stem cell support was planned. On January 1996 Cyclophosphamide 4 g/mq was infused and followed by stem cell harvest. After the first leukopheretic procedure a severe persistent thrombocytopenia occurred (plts < 15000/mm³) and daily platelet transfusions were required. Serum IgG antiplatelets antibodies were positive, bone marrow aspirate with increased megakaryocytes count was detected and diagnosis of ITP was made. Prednisone (75 mg/day) and intravenous Immunoglobulin (400 mg/kg for 4 days) was administered with partial remission (plts 10000/mm³). A double autotransplant procedure with stem cells support was performed with complete remission of M M. From June 1996 to February 1998, maintenance therapy (Interferon 3 MU, 3 times a week and Dexamethasone 20 mg on day 1 to 4 each months) was started, IgG level gradually increased, platelets count was unchanged. On April 1998 the platelet count fell under 50000/mm³; bone marrow citology showed increased megakaryocytes, plasma cell count was 6%. Prednisone 75 mg/day was started and platelets count rapidly increased to 143000/mm³. Prednisone was tapered and platelets count fell again to 27000/mm³. Prednisone was then associated with Azathioprine (100 mg/day) with partial recovery (plts 53000/mm³). On April 2000 disease recurrence occurred with anemia and serum IgG level increase to 3.7 g/dL. Thalidomide treatment (200 mg per day) and Prednisone (25 mg per day) were started. After 2 months of therapy IgG level fell to 655 mg/dL and platelet count rose to 206000/mm³. Prednisone therapy was stopped, platelet count remained unchanged. On April 2001 appearance of neurological...
toxicity (sensory polyneuropathy, WHO grade II) required Thalidomide reduction to 100 mg/day, platelet count slightly decreased to 150000/mm³. At present both Thalidomide dose and platelet count are unchanged. This patient was treated with specific therapy commonly used in primary ITP with only transient benefit. Thalidomide has anti-inflammatory, immunomodulatory and antiangiogenic effects; based on these findings has been used in several rheumatological and autoimmune diseases. ITP might be another autoimmune disease effectively controlled by Thalidomide. The T helper response has been classified in Th1 and Th2 type. Th1 cells can elicit delayed hypersensitive reactions and cell-mediated immune response, Th2 cells are important in suppressing cell-mediated immune reactions. As expected, Th1 phenotype is prevalent in patients with ITP. Thalidomide can switch T helper response from a Th1 to a Th2 type; the expansion of the Th2 type reaction, induced by Thalidomide, might explain its action on ITP. In conclusion, Thalidomide treatment was effective in this patient where MM was associated with ITP. Low dose Thalidomide (100-200 mg per day) could inhibit both myeloma cells growth and platelets destruction, obtaining long-term control of both diseases.

PU291
TREATMENT OF PEDIATRIC ANA-POSITIVE THROMBOCYTOPENIA
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The treatment of pediatric ANA-positive thrombocytopenia still remains a problem. The dose of steroids and the duration of therapy are tailored on an individual basis due to the lack of well-established guidelines. We retrospectively evaluated ANA-positive thrombocytopenic patients followed in our ward for prognostic factors and response to treatment with steroids administered at different dosages and tapered at different intervals to various maintenance dosages. Data were collected from medical charts of ten patients with median age 15 (range 8-17) years. Four of these patients (one male and 3 females) had the criteria for a diagnosis of systemic lupus erythematosus (LES), 2 of whom presented with diffuse proliferative glomerulonephritis, one patient with arthralgia, autoimmune thyroiditis, celiac disease and positive direct Coombs' test and the male patient with positive anticardiolipin IgM antibodies, arthralgia, oral ulcers, pericarditis and positive direct and indirect Coombs' tests. Two of the remaining 6 patients had familiarity for LES and 2 had arthralgia. At onset, PLT count was median 85000 (range 2000 - 149000). Severity of thrombocytopenia was distributed as follows: Grade 1 - four patients, Grade 2 - one patient, Grade 3 - two patients, Grade 4 - two patients. Three of the 4 LES patients had leukocytopenia ranging from 2400-3700/mm³ compared to 1 patient in the remaining group (p=0.01). The choice of the initial dose of prednisone (from 0.2 to 1.75 mg/kg) and the duration of full-dose therapy (mean 85, SD 8.5 days) were not associated with the grade of thrombocytopenia, nor with the presence of systemic symptoms. Time to remission of thrombocytopenia was mean 84, SD 7, days and was associated with the presence of systemic symptoms (p=0.09) and leukocytopenia (r= -0.57, p=0.05) and significantly dependent on the PLT count at onset (r=-0.80, p=0.04), while there was no association with the dosage and duration of prednisone. Prednisone was titrated in median 4 (range 2-6) days and 4 patients continued at a maintenance dose of 0.1 (range 0.05-0.12) mg/kg. In the 8 patients undergoing single therapy with prednisone, 6 had recurrence, though with higher PLT counts as compared to those at onset (p=0.05). The duration of remission was median 186 (range 116-256) days and was associated with the C3 level at onset (p=0.04) and seemed to be influenced by the dosage and the duration of full-dose prednisone and significantly by the duration of maintenance therapy (r=0.78, p=0.015) and its dosage (r=0.69, p=0.49).

In conclusion, the lack of guidelines for the treatment of ANA-positive pediatric thrombocytopenia induces the clinician to make arbitrary choices in treatment approaches. Recurrence is frequent though it does not seem severe. When prednisone is chosen as a single agent, dosage and administration protocols may influence the duration of remission. It is clear that future studies are necessary to reveal whether or not to treat these patients according to the presence or absence of systemic signs and to the grade of thrombocytopenia.
Essential thrombocytopenia (ET) and polycythemia vera (PV) are characterised by an imbalance between coagulant and fibrinolytic activities of plasma in which the pro-thrombotic activities predominates. To test this suggestion we measured key hemostatic proteins including, activated factor XII (FXIIa) as intrinsic activator, tissue factor pathway inhibitor (TFPI) as extrinsic activator, prothrombin fragment 1+2 (F1+2) as a indicator of thrombin generation, and thrombin activatable fibrinolysis inhibitor (TAFI) and d-dimer (DD) as a link between coagulation and fibrinolysis. We included 75 patients, 47 ET (19 men and 28 women, mean age 59 years) and 28 PV (17 men and 11 women, mean age 65 years), who fulfilled the PVSG criteria. Each parameter was measured using an enzyme immunoassay (ELISA) technique. The duration of disease ranged between 1 to 13 years. Of 75 patients, 47 ET; 22 PV received hydroxyurea, 4 ET were on interferon- alpha, 3 PV underwent phlebotomy alone and 21 (18 ET, 3 PV) were not receiving any cytoreduction. 66/75 (45 ET, 21 PV) received hydroxyurea, 4 ET were on interferon-alpha, 3 PV underwent phlebotomy alone and 21 (18 ET, 3 PV) were not receiving any cytoreduction. Of 75 patients, 47 ET (19 men and 28 women, mean age 59 years) and 28 PV (17 men and 11 women, mean age 65 years), who fulfilled the PVSG criteria. Each parameter was measured using an enzyme immunoassay (ELISA) technique. The duration of disease ranged between 1 to 13 years. Of 75 patients, 47 ET; 22 PV received hydroxyurea, 4 ET were on interferon- alpha, 3 PV underwent phlebotomy alone and 21 (18 ET, 3 PV) were not receiving any cytoreduction. 66/75 (45 ET, 21 PV) were on antiplatelet drugs. None of the studied patients had thrombotic risk factors. Both FXIIa and TFPI have been found to be lower (2.3±0.3 ng/mL and 65±3 ng/mL, respectively) (-0.062) and a significant correlation between TFPI and DD (p =0.024). On the basis of these results it is hypothesised that low FXIIa and TFPI reflect a consumption due to intrinsic and extrinsic coagulation activation generating high F1+2. This hemostatic status might operate for TAFI activation impairing fibrinolysis, as evidenced by the normal DD. These data suggest that a new coagulation measurement based on these hemostatic factors might be useful for better identifying thrombotic risk in ET and PV.

The role of angiogenesis in the progressive growth and metastatic process of tumors is well established, but the clinical prognostic significance of the angiogenic factors in malignant hematologic diseases is still not clear. In this study, we assayed serum levels of two major angiogenic factors, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) in 55 patients affected by chronic myeloproliferative disorders (CMD). Twenty-five of them were affected by essential thrombocytemia (ET), 10 by chronic myelocytic leukemia (CML), 14 by polycythemia vera (PV) and 6 by primary myelofibrosis (MF). These patients were compared to 55 healthy sex- age-matched subjects (control group). In all patients the VEGF concentration was significantly higher than in the control group (p<0.01). The highest concentration of VEGF, between the four groups of patients, was found in the subjects with ET (178.25±125.22 pg/mL, mean± standard deviation). Moreover, the VEGF levels were significantly higher in CMD patients with vascular complications than in CMD without complications (p<0.01). The b-FGF levels also appeared to be significantly higher in the CMD patients than in the control group (p<0.05). A significant correlation was found between the VEGF levels and the platelet count in the ET patients and the spleen index in the CML patients. VEGF level, in this study, was associated with an increased risk of thrombotic complications. In conclusion, we can affirm that there is an evidence of increased levels of soluble angiogenic factors in malignant hematologic disorders, therefore a high level of VEGF and/or b-FGF is a negative prognostic finding in this kind of pathologies.

Sequential Thalidomide and Erythropoietin in Idiopathic Myelofibrosis: A Successful Combination


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There is evidence of neoangiogenesis in idiopathic myelofibrosis (IMF). The stromal proliferation is a reac-
tive phenomenon caused by release of PDGF, TGFβ, bFGF, FGF and calcmodulin. Thalidomide retains antiangiogenic, immunomodulatory and cytokine regulatory properties suggesting a therapeutic role in IMF. Recombinant human erythropoietin (rHuEPO) has been used with moderate success in the treatment of transfusion-dependent anaemia secondary to IMF. A synergistic effect of the two drugs in IMF, although possible, has, up to now, not been shown. We thus treated one patient with IMF, previously insensitive to erythropoietin and scarcely responsive to thalidomide, used as single drugs, with a sequential combination of erythropoietin and thalidomide. Before the treatment the spleen size was 25 centimetres, hemoglobin: 7.4 g/L (requiring transfusion support of 2 unit packed red blood cells per month) and platelets: 45 × 10^9/L. A trephine biopsy showed a marked diffuse increase in reticulin fibers with hypocellular areas and coexistent hypercellular hemopoietic areas. Atypical megakaryocytes, granulocytic precursors, less than 5 CD34+ cells and erythroblasts were present. Thalidomide and rHuEPO have been used at the dosage of 100-600 mg daily and 150U/kg/day twice weekly, respectively. After 3 months of combined treatment anaemia and thrombocytopenia improved with transfusion independence, and 6 months later hemoglobin and platelets were respectively 15.5 g/L and 220 × 10^9/L. The spleen size reduced from 25 to 18 centimetres and then, after 5 months, to 12 centimetres. An increase of cellularity, with normal morphology of megakaryocytes and stable erythroblasts and granulocytic precursors, was histologically documented along with a slight decrease of reticulin fibers. Up to now, 22 months after the end of the combined treatment, the patient remains well, with normal blood counts and no further increase of the spleen size. Thus, the good response observed in our case suggests that thalidomide plus erythropoietin may be a option for IMF patients with an increased marrow fibrosis and a suboptimal marrow reserve or for those even with advanced disease, unresponsive to either agent used as single drugs.

PU295
PROGNOSTIC SIGNIFICANCE OF BONE MARROW HISTOLOGICAL DYSPLASTIC FEATURES IN ESSENTIAL THROMBOCYTHEMIA
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Essential thrombocythemia (ET) is characterized by a relatively favourable prognosis, but life-threatening complications adversely affect the clinical course in a substantial proportion of cases. In this work, we suggest that some histological parameters may help in defining prognostic scores that would allow the earlier identification of patients with ET at risk of severe complications. Bone marrow biopsies taken at diagnosis from 137 patients (M = 52, F = 85; median age 54 years) were reviewed according to Mihichls and Thiele (Int J Hematol 76:133;2002), and clinico-pathological relationships were looked for. A definite diagnosis of ET could be established in 55 patients. There were 51 patients with dysmegakaryocytopoiesis, 22 with myeloid hyperplasia, 45 with dyserythropoiesis, and 38 with dysmyelopoiesis. Moderate degree of reticulum thickening was observed in 29 cases. After a median follow-up of ten years, 32 patients had died and 52 had suffered a major event, which was thromboembolic in 27 cases and oncological in 25. The typical ET group had a lower incidence of oncological events (7 vs 18, p < 0.05) and better 25-year survival chances (overall survival: 72.83% vs 44.16%; event-free survival: 52.15% vs 14.69%; oncological event-free survival: 79.34% vs 43.5%). The patients experiencing an oncological event had significantly higher frequencies of dysmegakaryocytopoiesis (p < 0.05), myeloid hyperplasia (p < 0.01) and reticulum abnormalities (p < 0.001), and showed a trend towards higher frequencies of dysmyelopoiesis and dyserythropoiesis; they also had a significantly higher platelet count at diagnosis (p < 0.01) and included a borderline excess of male subjects. Higher frequency of dysmegakaryocytopoiesis was also observed in patients presenting a thromboembolic event. In conclusion only a minority of our patients perfectly satisfied the Mihichls & Thiele criteria for a diagnosis of ET; this group represents a subset of patients characterised by a more benign clinical course. Furthermore, we confirm (Haematologica 84: 17, 1999) that patients suffering major clinical complications show at diagnosis a significantly higher frequency of bone marrow dysplastic features.

PU296
MEYLOID BLAST CRISIS OF CHRONIC NEUTROPHILIC LEUKEMIA WITH Y CHROMOSOME LOSS AND TRISOMY 8: A CASE REPORT
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Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative syndrome characterized by sustained neutrophilia, marrow myeloid hyperplasia, hepatosplenomegaly, hyperuricemia, increased serum B12 level, high/normal leukocyte alkaline phosphatase (LAP) score and absence of the Ph chromosome and the BCR/ABL fusion gene. The clinical course is various. It is reasonable, today, to distinguish chronic stable CNL, CNL associated with plasma cell dyscrasia, CNL with...
myelodysplasia, and neutrophilic-chronic myeloid leukemia (N-CML). To date, few cases of CNL have been reported in the literature. We describe here a new case of CNL that evolved to myeloid blast crisis. A 77-year-old man was admitted in our medical unit on September 2001 because of a leukocytosis associated with mild hepatosplenomegaly and cutaneous bleeding. The peripheral blood showed Hb 70 grams/liter, PLT $62 \times 10^9/L$, WBC $29.8 \times 10^9/L$, $95\%$ neutrophils with dysplastic features, without granulocytic immaturity, LDH $321 \text{ U/L}$, uric acid $10.7 \text{ mg/dL}$, vitamin B12 $1360 \text{ pg/mL}$, LAP score $380$. The bone marrow aspiration showed marked myeloid hyperplasia ($85\%$) with dysplastic features, $4\%$ blasts; the karyotype was normal and the BCR/ABL translocation was lacking on RNA-PCR analysis. The patient was also suffering from ischaemic heart disease and aneurysm of the thoracoaorta. A diagnosis of CNL with dysplasia was made. Treatment with transfusions and hydroxyurea, which provided good WBC count control, was started. Sixteen months later the patient developed a marked leukocytosis (WBC $59.30 \times 10^9/L$) with $60\%$ myeloid blast positive for CD34, CD33, HLA-DR, and negative for CD11b, CD11c, CD14, CD15. Chromosome analysis revealed 46, X-Y,+8 (40/40 metaphase). Despite low-dose cytarabine treatment, the patient died one month later. Trisomy 8 and -Y are common chromosome abnormalities in CML blast crisis, as in de novo acute myelogenous leukemia, myelodysplasia, and myeloproliferative disorders. To our knowledge, this is the first case showing trisomy 8 with additional y chromosome loss in a CNL myeloid blast crisis. In spite of uncertain biological aspects of this disease, CNL, as others myeloproliferative disorders, may evolve to myeloblastic transformation. The CNL myeloid blast crisis has a poor prognosis.

PU297

EPIDEMIOLOGY OF MYELOPROLIFERATIVE DISORDERS

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All cases of hematologic malignancies newly diagnosed in Sardinian residents in the time period 1974-1993 have been collected, with an active survey, from the registries of clinical and pathology institutions during that time active in the island. Diagnoses’ validity was verified by consultation of patients’ clinical records and, for a part of cases, when needed, by discussion with attending physicians. Out of more than 7,000 collected cases of hematological malignancies, cases of Chronic Myeloproliferative disorders were 837(11%): Chronic Myeloid Leukemia (CML) 405, Polycythemia Vera(PV) 120, Essential Thrombocythemia (ET) 149, Methylfolicosis (MF) 111 and Unknown Type 52. For all of them incidence increases with age and demonstrates a male predominance which is less marked in CML and ET; this last disease is well represented also in younger age classes. Incidence of CML did not present substantial variation in the studied period, while PV,ET,MF in the first ten surveyed years were largely under-diagnosed (cases in first decade/cases in the second decade were respectively 31/89; 13/136; 40/71) possibly due to scarce awareness of the diseases and to reduced availability of the needed diagnostic tools (electronic blood cell counters capable of platelets counts, practices of bone marrow biopsy and red cell mass evaluation). In the following years the number of diagnosed cases increased, may be also inflacted by cases previously gone undiagnosed. For all four diseases, at the end of the studied period, age adjusted rates (World population) (Table 1) are comprised on the ample range reported for other western populations. Expected number of cases for 2001 were calculated for each disease by applying age- and sex-specific incidence rates of the whole 20 years period or, if there was a documented increase in incidence, of the last 10 or 5 years to expected Sardinian populations at 2001 (obtained from ISTAT) age strata by age strata, and by summing the results over the strata; they are reported in Table 1.

Table 1. Myeloproliferative disorders: age adjusted incidence rates and expected cases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age adjusted rates (world population) per 100,000 persons year</th>
<th>Expected cases in 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>CML (1974-1993)</td>
<td>1.30 (SE 0.08)</td>
<td>0.80 (SE 0.06)</td>
</tr>
<tr>
<td>PV (1984-1993)</td>
<td>0.54 (SE 0.07)</td>
<td>0.33 (SE 0.05)</td>
</tr>
<tr>
<td>ET (1984-1993)</td>
<td>1.08 (SE 0.14)</td>
<td>0.69 (SE 0.10)</td>
</tr>
<tr>
<td>MF (1989-1993)</td>
<td>0.34 (SE 0.06)</td>
<td>0.22 (SE 0.06)</td>
</tr>
</tbody>
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PU298

EPIDEMIOLOGY OF HEMATOLOGICAL MALIGNANCIES

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This study was undertaken to acquire knowledge of epidemiology of Hematological Malignancies (HM) in
Sardinian population, hitherto not yet studied. Principal aim was to obtain data useful for planning health care, but we were also interested in evaluating whether occurrence of these diseases was influenced by the peculiar genetic characteristics of our population and by the fact that only in relatively recent years Sardinian residents approached characteristics of the so-called developed populations. All cases of hematological malignancies newly diagnosed in Sardinian residents in the time period 1974-1993 have been collected, with an active survey, from the registries of clinical and pathology institutions during that time active in the island. Diagnoses' validity was verified by consultation of patients' clinical records and, for a part of cases, when needed, by discussion with attending physicians. For each disease we calculated incidence rates and looked for eventual presence of temporal changes. Incidence rates of most HM show an increase with increasing age and a male predominance, except for acute lymphoblastic leukemia with its typical childhood peak and for Hodgkin's disease with the excess of nodular sclerosis in young adult females. Absolute number of new diagnoses more than doubled during the 20 studied years (Table 1), only in part as consequence of ageing of population. Incidence rates of most hematological malignancies increased during the studied period, exception made for Hodgkin disease, chronic myeloid leukemia, and acute lymphoblastic leukemia. The observed increase of incidence seems to consist of two different components. One is a fictitious increase, possibly due to an easier access of people to the health care system that moreover, during the studied time, acquired better diagnostic efficiency. It was of particular relevance for chronic lymphocytic leukemia, multiple myeloma, and some myeloproliferative disorders and was much less evident after 1984-1989. The other one is a true increase, consequence of occurrence of more cases of the diseases, as happened in Non Hodgkin's lymphomas and similarly, although to a lesser extent, in myelodysplasias and acute myeloid leukemia. Its importance in other diseases may have been obscured by the confounding effect of the changes in global diagnostic efficiency of Sardinian health care system in this field.

Age and sex distribution and age adjusted rates of all hematological malignancies, at the end of studied period, are similar to those reported in other western populations: therefore these data do not document that the genetic peculiarities of Sardinian population have had an influence on the occurrence of these diseases. The risen incidence has largely increased the burden charged by hematological malignancies to health care system, still more due to the increased number of aged people. This fact must be taken into account in the training of hematological staff, who must be prepared to face not only hematological problems but also problems of the degenerative diseases typical of oldest ages. Expected cases for 2001, exposed in Table 1, were calculated for each disease by applying age- and sex-specific incidence rates of the whole 20 years period (or, if there was a documented increase in incidence, of the last 10 or 5 years) to expected Sardinian populations at 2001 (obtained from ISTAT), age strata by age strata, and by summing the results over the strata.
PU299
A CASE OF AGGRESSIVE MASTOCYTOSIS WITH INVOLVEMENT OF BILIARY DUCTS SIMULATING SCLEROSING CHOLANGITIS
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A 43-year-old woman was admitted to hospital in February 2002 because of abdominal pain, diarrhoea, fever, flushing, weight loss, hepatosplenomegaly, ascites of transudative origin. White blood cell count 5.900/mm³, hemoglobin 11 g/dL, platelet count 186,000/mm³, total serum bilirubine 1.0 mg/dL (normal 0.2-1.0), alkaline phosphatase level 497 U/L (normal 32-122), γ-glutamyltransferase level 77 U/L (normal 7-50). The bone marrow biopsy showed increased cellularity and monocytosis. Endoscopic retrograde cholangiopancreatography showed reduced biliar ducts. Percutaneous liver biopsy diagnosed primitive sclerosing cholangitis. The patient was treated with steroids with improvement of her general condition. The patient was first seen in our hospital on May 2002 because of worsening of her conditions and hepatic stasis indexes despite increase of steroids. She presented fever, flushing, abdominal pain, ascites, hepatosplenomegaly. White blood cell count 3.000/mm³ with normal differing, abdominal pain, ascites, hepatosplenomegaly.

PU300
POLYMORPHONUCLEAR LEUKOCYTE-PLATELET AGGREGATES IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA: ROLE OF PMN ACTIVATION
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ET and PV are chronic myeloproliferative disorders characterized by a high incidence of thromboembolic complications. We have previously demonstrated that PMN from ET and PV patients circulate in an activated status. In this study we wanted to evaluate whether activated PMN may have increased interactions with platelets leading to high circulating levels of PMN-platelet aggregates, as found in other several clinical conditions at high thrombotic risk. In ET and PV subjects, we have measured the levels of PMN/platelet aggregates and tried to assess the relative contribution of PMN activation (evaluated by CD11b expression) and/or platelet activation (evaluated by CD62P expression) to PMN-platelet aggregates generation. Whole blood samples from 43 patients with ET, 30 with PV and 50 control subjects (C) were studied in both basal condition and after in vitro f-MCP stimulation. PMN-platelet conjugates were analyzed by flow cytometry and defined as the percentage of CD11b⁺PMN co-expressing a platelet-specific marker (i.e. CD42b or CD62P). The results confirmed PMN activation in vivo.
as shown by significantly (p<0.01) higher CD11b levels on PMN from ET (167±10 MFI) and PV patients (160±11 MFI) compared to controls (101±8 MFI). The CD11b/CD42b aggregates were increased in ET and PV (50±4% and 38±5%, respectively) compared to controls (23±3%; p<0.01), as it were the CD11b/CD62P aggregates (ET=28±4%, PV=19±3% and C=13±4%; p<0.01). Analysis of platelet surface showed no variations in CD42b expression, whereas increased CD62P levels, a surface antigen specifically exposed on platelet activation, were expressed by ET (7.7±0.9%) and PV (9.1±0.8%) platelets, compared to controls (5.4±0.7%). The f-MLP-induced in vitro PMN activation increased CD11b levels by PMN, and simultaneously increased CD11b/CD42b aggregates in samples from all groups, and CD11b/CD62P aggregates only in ET and control groups. No significant differences in platelet CD62P and CD42b levels were found after in vitro f-MLP stimulation in any group. In conclusion, in ET and PV patients increased levels of PMN/platelet aggregates were found in association with the occurrence of activation in vivo of both PMN and platelets, showing that mixed aggregates formation may reflect qualitative abnormalities of both cells. However, a major role for PMN in increasing the leukocyte/platelet interactions is suggested by the in vitro results.

PU301
CHRONIC CYTOPENIAS RELATED TO AUTOIMMUNE MYELOFIBROSIS

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Autoimmune cytopenias, frequently due to cell-binding immunoglobulins with complement-dependent or independent destruction of peripheral blood cells, may also be referred to additional mechanisms including bone marrow dysfunction related to humoral or cellular immune reactions targeting hemopoietic and/or stromal cells. We report on three patients admitted to our Department for chronic cytopenias; two of them showed leukopenia with neutropenia and anemia, and the other one thrombocytopenia and anemia. Furthermore, we found bone marrow increased reticulin fiber content, hypocellularity (1 case) and normocellularity (2 cases), no clustered megakaryocytes, reactive lymphoid infiltration, lack of significant teardrop poikilocytosis and leukoerythroblastosis on peripheral blood smears, absence of eosinophilia or basophilia, normal sized spleen, positive autoimmune serology. The diagnostic work-up resulted in clinicopathological patterns consistent with autoimmune myelofibrosis associated with concomitant Sjögren’s syndrome and Hashimoto’s thyroiditis in a patient whereas only anti-nuclear mitotic apparatus protein autoantibodies were detected in another one, who did not develop any associated autoimmune disease during a 46 month follow-up; the third patient had been diagnosed as having Sjögren’s syndrome one year before admission. Autoimmune myelofibrosis is an emerging entity, that recognizes primary immunopathogenetic mechanisms and results in various degrees of isolated or combined chronic peripheral blood cytopenias. It was prominently described in patients affected by systemic lupus erythematosus, and more recently also in those with autoimmune laboratory and/or clinical manifestations not fulfilling the classification criteria of a well defined autoimmune disease. In our patients, other disorders known to cause bone marrow fibrosis could be excluded. The most relevant differential diagnosis involved chronic idiopathic myelofibrosis, which discloses autoimmune clinical and laboratory features in a proportion of cases. We conclude that autoimmune myelofibrosis has to be taken into account as a distinct clinicopathological entity in the diagnostic evaluation of patients presenting chronic cytopenias.

PU302
ANAGRELIDE TREATMENT FOR THROMBOCYTOSIS IN MYELOPROLIFERATIVE DISORDERS

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Anagrelide is an oral imidazoquinazoline drug effective in thrombocytosis associated with chronic myeloproliferative disorders (CMPD). It decreases platelet production by interfering with the post-mitotic maturation of the bone marrow megakaryocytes. Adverse effects are primarily caused by the drug's direct vasodilating effects and may limit the use of the drug. In this study we report the our experience with the use of anagrelide (efficacy and toxicity) in a cohort of MPD patients. Patients. Sixteen patients (4 males, 12 females, median age 53 years) with thrombocytosis for CMPD (13 with ET, 2 with PV, one with IMF) were treated with anagrelide 1-2 mg/day (median follow up on anagrelide treatment: 8 months, range 3-15). The median pre-treatment platelet (plt) count was 788,000/µL, range 618,000-1,289,000; indications for cytoreductive treatment were: previous thrombosis or symptoms of the microcirculation refractory to aspirin associated with other risk factors for thrombosis. Results. 12/16 patients (75%) achieved a complete sustained response (plt count < 600,000/µL) by
Essential thrombocytemia (ET) is a chronic myeloproliferative disorder characterized by a long median survival, but serious complications such as acute hemorrhagic or thrombotic events are frequent. Few informations are available about risk factors for developing ET, because of lack of epidemiological studies focussed on this item. In order to analyze the possible association between ET and some occupational and environmental risk factors we conducted a case-control study. 93 patients were enrolled in two hospitals, in a restricted urban area (Turin-Italy), and 280 subjects randomly selected from the general population as a control were also included in the analysis. We found an association between ET and selected occupations. OR estimates suggest a significant association between ET and cooks/waiters (OR 5.10, CI 1.59-16.99; p<0.05) and non significant association between ET and hairdressers, farmers, electricians were considered at risk of developing ET.

PU304
THROMBOPHILIA SCREENING IN ESSENTIAL THROMBOCYTtemia: A REPORT OF FIFTEEN CASES
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Essential Thrombocytemia (ET) is a chronic myeloproliferative disorder complicated by high incidence of thrombotic events. Thrombosis may be fatal or may induce permanent disability. Factors associated with risk of thrombosis are advanced age and a previous thrombotic event. The indication for reduction of platelet count in ET patients is still disputed, usually patients start a specific therapy on the basis of the platelet number, age and thrombotic complications. The role of inherited or acquired coagulation abnormalities in the pathogenesis of thrombosis in ET patients is not well established. We explored the presence of thrombophilic factors valuing the level of natural anticoagulant such as antithrombin III (ATIII), protein C (PC), protein S (PS), plasma homocysteine (HC), IgM and IgG anticardiolipin antibodies (aCL), IgM and IgG anti-beta2-glycoprotein antibodies, polymorphism of clotting factor II (G20210A) and factor V Leiden in patients with essential thrombocytemia. From April 2002 to May 2003 we analyzed 15 ET patients (7 male and 8 female, mean age 66.5 years). All patients had a platelets value < 1.000.000x10^3 µL, were taking low dose aspirin, had no previous thrombotic complication but referred thrombotic events in their family history. Of 15 patients 7 (46.7%) showed haemostatic abnormalities: 1 patient (6.7%) had a PC deficiency, and 6 patients (40%) had high plasma HC. In the group of patients with high plasma HC 1 patient was heterozygous for factor V Leiden and had high levels of anticardiolipin IgM and 1 patient had high levels of anticardiolipin IgM and IgG. All patients had normal vitamin B12 and folate plasma values. Of 6 patients with high plasma HC 1 had thrombophilic complications. The frequency of HC high values observed in our patients is higher than the frequency described in the literature within the general population. However, our study is not conclusive as a longer follow-up and a large cohort of patient are needed. The definition of thrombotic risk in ET patients is still controversial and a thrombophilic test utility should be further evaluated.
PU305
SEVERE LIFE-THREATENING HYPOCALCEMIA AFTER BISPHOSPHONATE ADMINISTRATION IN AGNOGENIC MYELOID METAPLASIA
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In November 2002, a 72-year old man was admitted to our hospital because of serious hypocalcemia. The patient had well-documented agnogenic myeloid metaplasia (AMM) diagnosed two years previously. He was then referred to a radiation and cancer therapy Center and was treated on June 2002 with 3200 rads to the spleen. The treatment induced a good reduction of splenomegaly with a transient, severe neutropenia. When admitted the patient reported profound asthenia; WBC, Hb, and PLT were 6.4 x 10^9/L, 101 gm/L, 95 x 10^9/L, respectively. Calcemia was unbelievably low: 0.75 mMol/L (n.v. 2.15-2.62 mMol/L), ionized calcium was 0.68 mMol/L (n.v. 1.12 - 1.32), on ECG Q-T was elongated (0.42 sec. instead of 0.35). Only subsequently we knew that the patient had been treated with a new bisphosphonate, zaledronic acid. The rescue of calcemia to values near normal range required about two weeks during which great amounts of calcium gluconate i.v. and calciferol were administered. Parathyroid glands function was assessed and resulted in elevated, compensatory, parathyroid hormone production. The conclusion was of serious hypocalcemia due to bisphosphonate administration. The follow-up of the patient has shown a difficult control of calcemia and deficiency of vitamin D. Six months after the administration of zaledronic acid had been stopped, both oral calcium and D vitamin supplementation are still required. This case illustrated some more investigations are needed to validate the use of bisphosphonate in AMM have been published. Some more investigations are needed to validate the opportunity of the utilization of zaledronic acid in this disease. In AMM the mechanism of bone involvement is quite different from metastatic bone disease so it is possible to suppose that bisphosphonates can permanently modify calcium bone metabolism due to erosion and resorption by osteoclastic activity. Finally, a more careful and closer follow-up of calcemia is certainly required in patients with AMM treated with bisphosphonates.

PU306
IMATINIB-MESYLATE (GLIVEC) THERAPY OUTSIDE THE SETTING OF BCR/ABL POSITIVE HEMATOLOGICAL MALIGNANCIES: THE CASES OF HYPEREOSINOPHILIC SYNDROME, SYSTEMIC MASTOCYTOSIS, IDIOPATHIC MYELOFIBROSIS AND ACUTE MYELOID LEUKEMIA
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Here we report eleven patients with BCR/ABL negative hematologic malignancies characterized by the possible involvement of alternative genes with increased tyrosine-kinase activity (such as c-KIT or PDGF-R mutants), we recently treated with the selective tyrosine-kinase inhibitor imatinib-mesylate (Glivec, Novartis-Pharma). Four patients were affected by Hypereosinophilic Syndrome (HES). In a 64-year-old man with moderate pruritus (WBC count 19.500/mm³, eosinophils 63%), bone marrow examination revealed a clonal myeloproliferative disorder with 40% of cells belonging to the eosinophilic lineage. Cytogenetic and FISH studies evidenced a t(2:4) translocation. This patient received imatinib-mesylate at the dose of 100 mg/d, achieving a rapid response in a few days. After five months, he is asymptomatic and in complete morphologic and cytogenetic remission. The current dose of imatinib is 100 mg on alternate days. The second HES patient was a HCV-positive 64-year-old female, who presented with marked pruritus and cutaneous nodular infiltrates. WBC count was 30.100/mm³ (eosinophils 58%). Marrow cellularity showed massive eosinophilic infiltration, without karyotypic abnormalities. This patient did not respond to the starting dose of 100 mg/d, but two weeks after the increase of imatinib up to 400 mg/d, the WBC count normalized, while skin lesions and pruritus disappeared. After 4 months of therapy, however, a new increase in eosinophil count occurred. A dose of 600 mg/d is currently given. Two additional patients with HES are too early for evaluation of response to imatinib-mesylate. The presence of the FIP1L1/PDGFRA fusion gene is under investigation in all these patients. A 33-year-old male with sporadic systemic mastocytosis (SM) received Glivec at the dose of 400 mg/d. He had typical diffused skin lesions, abdominal pain and diarrhoea. Histologic evidence of diffuse mastocytic infiltration was documented at level of the skin, stomach, intestine and bone marrow. Karyotype was normal. Neither improvement of the clinical symptoms, nor reduction of cutaneous, gastro-intestinal and marrow mastocytic infiltration (as evaluated by sequential tissue biopsies) were observed after 16 weeks of therapy. Imatinib-mesylate (200-600 mg/d for 8-16 weeks) was given to 3 patients affected...
by Myelofibrosis (M F) (two males, one female, aged 61 to 73). One of them had a post-polyctenia vera disease. All patients had marked splenomegaly, one had concomitant hepatomegaly. Two patients were transfusion-dependent. No relevant benefit was observed in these patients. Three mult-treated c-KIT positive acute myeloid leukemias (AML) (two males, one female 55 to 77-year old) received imatinib-mesylate at the dose of 400-600 mg/d for at least 12 weeks. Two of them had a post-myelodysplasia disease. In one patient a decrease of marrow blasts from 30% to 10% and a reduction of c-KIT expression on the surface of leukemic cells were seen. This patient died of causes not related to AML. In the other cases no response was observed. We confirm the sensitivity of HES to Glivec, although different dosages may be required to achieve response. On the contrary, our data do not support the efficacy of imatinib in sporadic SM and M F. The partial effects seen in a case of AML require further investigations.

PU307
CIRCULATING CD34+ CELL COUNT IS HIGHER IN MYELOFIBROSIS SECONDARY TO ESSENTIAL THROMBOCYTENIA AND POLYCYTENIA VERA THAN IN IDIOPATHIC MYELOFIBROSIS

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The circulating myeloid progenitor pool is increased in Myelofibrosis with Myeloid Metaplasia (MMM) and may be quantified by measuring the peripheral blood (PB) CD34+ count. Previous studies have demonstrated that absolute number of CD34+ circulating cells is an useful parameter to discriminate MMM from other chronic myeloproliferative disorders. In fact PV and ET do not present any increase in circulating CD34+ cells, on the contrary higher levels of circulating CD34+ is considered an index of myelofibrosis. After several years of chronic phase, some patients with ET and PV may develop marked bone marrow fibrosis along with myeloid metaplasia of liver and spleen. This stage of the disease is known as post-polycitemic myelofibrosis (PPV-MM) and post-thrombocytemic myelofibrosis (PET-MM) respectively, clinical and laboratory presentation is similar to idiopathic MMM, except for higher frequency of chromosome abnormalities. To evaluate eventual relationship between myelofibrosis and PB CD34+, we compared PB CD34+ count in patients with PPV-MM and PET-MM with idiopathic MMM patients. We analyzed at time of diagnosis peripheral blood samples from 9 patients with idiopathic MMM and 7 patients with PET-MM or PPV-MM, between January 1999 and February 2003. Absolute number of CD34+ circulating cells was determined by flow cytometry, using a modified MILAN PROTOCOL. Median absolute number of CD34+ circulating cells in PET-MM and PPV-MM was significantly higher than in idiopathic MMM: 124.7×10⁶/L (range 0.3-1.087,8×10⁶/L) in PET-MM and PPV-MM, versus 38.9×10⁶/L (range 2.7-120.1×10⁶/L) in idiopathic MMM. Also median percentage of CD34+ cells was higher in secondary MMM (1.31%, range 0.01-3.93%) compared to idiopathic MMM (0.56%, range 0.04-1.56%). Finally, median absolute leukocyte count (WBC) was higher in secondary MMM (10.580/µL, range 3.350-49.220/µL) than in idiopathic MMM (7.410/µL, range 670-21.800/µL). Only 2/9 (22.2%) patients with idiopathic MMM showed CD34+ count > 100×10⁶/L, versus 4/7 (57.2%) patients with myelofibrosis secondary to ET or PV. These findings may suggest that PB CD34+ count is higher in secondary MMM compared to idiopathic MMM; considering that ET and PV normally present a lower count of PB CD34+, an increase of this count may suggest an evolution in secondary MMM.

PU308
PORPHYRIA CUTANEA TARDA IN A PATIENT WITH MYELOFIBROSIS

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Porphyria cutanea tarda (PCT) is a metabolic disorder of heme biosynthesis, characterized by reduced uroporphyrinogen decarboxylase (URO-D) activity and increased urinary excretion of porphyrins. It is usually an acquired condition caused by inhibition of the URO-D enzyme in the liver (sporadic or type I PCT), while a minority (up to 20%) of patients have an inherited deficiency of URO-D (familial or type II PCT). PCT is characterized clinically by a photosensitive dermatosis associated with skin fragility and blistering. Clinical manifestations are often precipitated by triggering factors such as iron, hereditary hemochromatosis, hepatitis C virus infection, alcohol abuse, estrogens, a family history of PTC, solid tumors and hematologic malignancies. Association with lymphoma, chronic lymphoid and myelogenous leukemia and myelodysplastic syndrome has been reported, while association with Myelofibrosis (MF) is extremely rare and only one case, to our knowledge, has been described. Current treatment of PCT includes phlebotomy, low-dose chloroquine and the combination of both regimes. The therapeutic action of phlebotomy and chloroquine is, mainly, mediated by the reduction of hepatic iron stores. In fact,
Iron may inhibit URO-D activity by fostering the production of oxygen radicals that attract catalytic sites of the enzyme. In recent investigation has been found that oral Thalidomide at dose of 3 mg/kg/day, led to clinical recovery and complete biochemical remission. The clinical recovery of cutaneous vesicles and fragility is probably, at least partially, associated with the anti-inflammatory properties of oral thalidomide and, theoretically, with an increased in URO-D transcription or an allosteric action of thalidomide on this enzyme. We report a rare case of type II PTC associated with M.F. A 56-year-old female, with a 2-years history of MF and treated conservatively with frequent packed red blood transfusions developed the intermittent appearance of blisters on the dorsum of her both hands. These lesions form a scab and disappear after a few weeks, with new blisters returning in an episodic manner. Skin biopsy revealed a subepidermal vesicle with scanty cellular infiltrate. The possibility of porphyria cutanea tarda was raised and the diagnosis was subsequently confirmed with a porphyria screen that showed positive urinary porphyrins (6796.00 μg/24h-n.v. 0.00-240.00) and negative urinary porphobilinogen. Moreover low URO-D activity levels in red blood cells (1.96 nmol/copro/h/mg prot-n.v. 5.7±0.7), evaluated by HPLC analysis and mutation 425 G→A (R142Q) in the 5 exon (homozygous) were detected. Serum Ferritin was 1300 micrograms/L. Hereditary hemochromatosis and HCV infections were excluded. The patient denied any recent exposure to chemicals or toxins and tobacco, estrogens and alcohol use. She wasn’t taking any medication. A diagnosis of PTC was made and treatment with oral Thalidomide at dose of 300 mg/day for 1 week and 200 mg/day for three weeks was performed. After three weeks of treatment the patient lamented for drowsiness, intermittent constipation, lower limbs’ oedema and dry mouth. Moreover new vesicle and bullae on feet were observed, so the treatment with Thalidomide was stopped. The patient was treated with chloroquine at a dose of 150-mg po twice weekly and with desferoxamine at a dose of 2 g/day. Phlebotomy was not appropriate, given her low hemoglobin level. Already after the first month of treatment no new vesicles and/or bullae could be observed and there was a rapid decrease in the urinary total porphyrins excretion. This case supports the hypothesis that development of PCT in patients with hematologic disorders is more than casual, but may be provoked by several factors (exogenous and endogenous). In our case, both genetic and environmental factors contribute to the pathogenesis of PCT. Myelofibrosis, given the frequent blood transfusions requirement, may play an important role in the triggering of PCT. High ferritin level, in fact, is known as unmasking factor the symptoms of PCT.

The optimal management of Essential Thrombocythemia (ET) during pregnancy is an important issue. Obstetric complications include spontaneous abortion in the first-trimester; maternal complications, both hemorrhagic and thrombotic, are rare but more common than those seen in normal pregnancy. We report, here, three case reports of ET in pregnant women. Patient #1. The patients was 28 years old woman who presented in October 2000 with ET. She was symptomatic (mild paresthesias and severe headache). The platelets count was 865×10^9/L and was reduced by recombinant Interferon- α at 3×10^4U subcutaneously every other day. The patient became pregnant in September 2001 and she spontaneously stopped the treatment. At that time the platelet count was 696×10^9/L. A low dose aspirin (100 milligrams/day) was started. The pregnancy was uncomplicated and the platelet count gradually decreased until the seventh month to a plateau of about 500×10^9/L. In June 2002 she delivered a healthy full-term female infant without hemorrhagic or thrombotic complications. At that time Aspirin was stopped and prophylaxis of deep venous thrombosis with low-molecular-weight heparin was started for seven days. After delivery the platelet count gradually returned to pre-pregnancy levels (894×10^9/L). Patient #2. The patient was a 30 years-old woman who was diagnosed with ET in February 1999. She was asymptomatic and the platelets count was 850×10^9/L. She became pregnant in May 2002. No specific therapy was administered. The pregnancy ended in spontaneous abortion in the first trimester. Patient #3. On June 2000 a 25 years old woman was referred to our hospital for thrombocytosis. According to the criteria of Polycythemia Vera Study Group a diagnosis of ET was made. She was treated with Aspirin (100 mg/day) for severe headache (Plt 650×10^9/L). She became pregnant in August 2002. Aspirin was continued during the pregnancy. The platelet count was lower during this time (PLT 480×10^9/L). In May 2003 the patient delivered a healthy female infant by cesarian section. In conclusion, our limited experience, in accordance with other authors, suggests that: i) a positive impact of low-dose Aspirin during pregnancy. This treatment was correlated with a favourable outcome (live birth versus abortion) when compared to no treatment; ii) a significant decrease in platelet count during pregnancy in ET has been observed. Nevertheless, prospective studies are necessary in order to establish the best therapeutic approach in this subgroup of patients with ET.
PU310

CUTANEOUS LESIONS ASSOCIATED WITH HYDROXYUREA THERAPY IN MYELOPROLIFERATIVE DISORDERS

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From January 1991 to December 2000, 210 consecutive patients undergoing Hydroxyurea therapy for myeloproliferative disorders (chronic myeloid leukemia, polycythemia vera, primary thrombocytethemia, agnogenic myeloid metaplasia) were studied for skin lesions at the U.O. Ematologia in Cagliari. Hydroxyurea dose varied between 10-30 mg/kg. The period of treatment ranged between six months and nine years. None of patients had medical conditions associated with cutaneous lesions. Results: Thirty out of 210 patients (14.3%) developed the following lesions. These included melanonychia in 9 patients (4.3%), leg ulcers in 18 patients (8.5%), epitheliamias in 4 patients (1.9%) and dermopathy (localised acral erythema, lichen-like dermatitis, dermatomyositis-like erythema) in 10 patients (4.8%). Lesions occurred in the lower extremities (legs, hands, feet) and in the light exposed areas. All patients who developed skin lesions were taking the maximum Hydroxyurea dose (30 mg/kg) and lesions developed in few months. Discontinuation of therapy lead to the healing in about three months. Also in patients with epitheliamias discontinuation was effective but it was coupled with surgical treatment. Only in one patient the neoplasm developed very rapidly and destructively, producing severe mutilations of face and scalp and finally fatal. The mechanism underlying the genesis of these entities are yet to be defined. These include: Inhibition of the actively dividing cells of the epidermis, damage of the basal layer of the skin by reduction in oxygen requirements for proliferation, because of megaloblastic change of the erythrocytes and finally inhibition of DNA repair function, reduced proliferation of basal cells, chromosomas damage. Cutaneous lesions are common side effects of Hydroxyurea therapy, patients should be controlled for this complication.

PU311

ATYPIC MYELOPROLIFERATIVE DISORDER WITH T(8;13): CASE REPORT


In the group of chronic myeloproliferative syndromes is possible to identify some aggressive forms characterized by a reciprocal translocation that disrupts specific tyrosin kinase genes that deregulate haemopoiesis in a manner analogous to BCR-ABL. Recently has been identified one of the genes most commonly involved in these translocations, FGFR1, situated on the band 8p11. Disruption of FGFR1 is associated with a disease entity known as the ‘8p11 myeloproliferative syndrome (EM S)/stem cell leukemia lymphoma syndrome (SCLL)’, an aggressive chronic myeloproliferative disorder that frequently presents with eosinophilia and associated T-cell lymphoma, with fatal outcomes. The four partner genes currently identified are located on chromosome 6, 9, 13 and 22. All these translocations result in the production of constitutively activated tyrosine kinases fusion proteins. The disease appear to be ineradicable by conventional chemotherapy, although some patients have apparently been cured by allogeneic bone marrow transplantation. In November 2002 a 48-year-old woman presented with leukocytosis (WBC 20,000/mm³), moderate eosinophilia, mild splenomegaly; she was otherwise in good health. She underwent bone marrow aspirate which showed only myeloid hyperplasia. Cyto genetic analysis showed a 46,XX, t(8;13)(p11;q12) karyotype. PCR probe for BCR/ABL translocation did not show the BCR rearrangement. The location of the breakpoint on chromosome 8 was confirmed with fluorescence in situ hybridization using a chromosome 8 centromeric probe. With a specific probe we demonstrated the presence of a new fusion gene FGFR1-ZNF198. The patient developed rapidly progressive generalized lymphoadenopathy with a peculiar tonsillar hypertrophy. Tonsillar biopsy and cervical lymphonode microbiopsy showed reactivity for CD2, CD5, CD7, TdT. The cells were negative for CD34. This analysis supported a diagnosis of T lymphoblastic lymphoma. Bone marrow biopsy and cytfluorimetry otherwise confirmed only myeloid hyperplasia, but no evidence of a lymphoblastic population. At the moment the patient is being treated with aggressive chemotherapy according with a LBL regimen with good clinical response.
HEMATOLOGIC MALIGNANCIES AND SOLID TUMORS IN 305 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA


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Background. Essential thrombocythemia (ET) is often considered a benign disorder with an overall survival similar to that of the general population. The natural evolution of the disease to leukemia is rare and this may be potentiated by the use of chemotherapeutic agents including hydroxyurea, by far the most used drug presently. Illustrating the experience of our hematologic department in secondary tumors developed in patients with ET. Methods. We retrospectively reviewed a consecutive cohort of 305 patients (197 women, 108 men) with ET diagnosed between 1977 and 2002. Follow-up data were obtained from case files and were registered until December 2002. The median age at diagnosis was 58 years (range 18-87). The median follow-up was 8 years (range 1-23). The platelet counts at the first presentation varied from 489 to 3,200×10⁹/L (median 843). Patients treated with chemotherapy were 174/305 (57%). By December 31th 2002 64 patients were died, 48 were lost at follow-up and 193 were alive. Results. Sixteen percent of the patients treated with chemotherapy developed a second tumour versus 7.6% in the group not treated (hematological malignancies 4.6% versus 2.3% respectively). Thirteen patients had an hematologic malignancy: 8 acute myeloid leukemia (3 AML-M7), 2 lymphoblastic leukemia, 3 non-Hodgkin’s lymphoma (NHL). In this cohort of patients (6 women, 7 men), aged from 39 to 81 years, chemotherapy was given to eleven patients (85%); hydroxyurea, another drug 6, other drugs 5. Median time from ET diagnosis and leukemia/NHL and from the beginning of chemotherapy and leukemia/NHL were respectively 82 months (range 36-174) and 84 months (range 5-160). All patients except one died of progressive disease. Twenty-six patients had a solid tumour: 9 gastrointestinal tract, 7 lung (all smokers), 3 kidney, 2 prostate, 2 urinary bladder, 1 breast, 1 larynx, 1 leiomyosarcoma, 1 thymoma. Interval time between ET diagnosis and solid tumour ranged from 23 to 184 months. Seventy percent of these patients were treated with a myelosuppression: hydroxyurea 11, another drug±hydroxyurea 10. By December 2002 8 patients were alive, 2 patients were lost at follow-up, 16 patients died of progressive disease and its complications. In one patient with larynx cancer the final cause of death was a stroke. Conclusions. Prevalence rate of secondary malignancies is higher in the group of patients treated with myelosuppression. Eleven out of thirteen patients with hematologic malignancies received long-term therapy (> 4 years) and this may have contributed to the transformation. In the cohort of patients with solid tumour, the rate of patients treated was less (70% versus 85%), the median duration of myelosuppression was similar (50 vs 55 months) but other risk factors may have contributed to the development of the malignancy (i.e. smoking). These data need to be confirmed by controlled trials. Although myelosuppression with hydroxyurea reduces the risk of thrombotic complications, its use must be cautious in the low-risk patient especially if young due to its potential leukemogenicity.

DEVELOPMENT OF ACUTE LEUKEMIA IN A PATIENT WITH ESSENTIAL THROMBOCYTHEMIA TREATED WITH HYDROXYUREA

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Introduction. Essential thrombocythemia (ET) is a myeloproliferative disorder, which is thought to develop from a multipotent stem cell. In fact, cytogenetic and immunocytochemical studies indicate a trilineage involvement of the bone marrow. ET is characterized by thrombocytosis and clinically by vasomotor symptoms, thrombohemorragic complications and recurrent fetal loss. ET rarely evolves into AML, sometimes preceded by myelodysplastic syndrome. Such transformation mostly occurs in patients treated with alkylating agents, especially busulfan or radiophosphorus. So actually hydroxyurea has emerged as the treatment of choice in patients with ET and a high risk of thrombosis (age > 60 years, platelets count > 1,500×10⁹/L or previous thrombosis event) because of its efficacy and only rare acute toxicity. Case report. We report a case of leukemic evolution from ET, preceded by a myelodysplastic syndrome, in a 71-year-old man, affected by ET since October 1999. At diagnosis he presented pruritus, splenomegaly, elevated levels of platelets (1,500×10⁹/L), many platelets and megakaryocytes at bone marrow biopsy, in absence of other causes of platelets increase. So, he was treated with aspirin and hydroxyurea. In May 2000, platelets count was about 12 ×10⁹/L, so he interrupted hydroxyurea therapy. In October 2002, blasts appeared in peripheral blood; bone marrow biopsy showed a percentage of CD33+ myeloid cells superior to 90%. After low dose AraCytin and 6-Mercaptopurine therapy, he was submitted to monoclonal antibody anti-CD33 therapy (Gemtuzumab-Ozogamicin), but blasts were always present in peripheral blood. In January 2003 the patient died from cardiocirculato-
A CASE OF IDIOPATHIC MYELOFIBROSIS WITH PURE RED CELL APLASIA AND THYMOMA: SUCCESSFUL TREATMENT WITH CYCLOSPORIN-A

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Background. Idiopathic myelofibrosis with pure red cell aplasia is an unusual form of idiopathic myelofibrosis, which is considered a distinct functional and clinical entity. Its pathogenesis is probably autoimmune. Case report. We report the case of a 69-year-old man who presented with severe anemia and weakness nine months go. Three months earlier he underwent successful surgical excision of a thymoma, which is still in complete remission. The patient had pale skin and splenomegaly (17 cm in length as measured by ultrasonography). He had severe anemia (hemoglobin, 4g/dL; mean corpuscular volume, 78,5 fL; reticulocytes, 9.300/µL), with normal white blood cell (4.500/µL) and platelet counts (400.000/microliter). Examination of a peripheral blood smear showed anisopoikilocytosis with rare teardrop red cells. Serum lactate dehydrogenase was normal, while serum ferritin was increased (927 ng/mL; normal range: 30–400); direct and indirect antiglobulin test, antinuclear, antimitochondrial and anti-smooth muscle antibodies were negative. The morphological examination of a bone marrow biopsy revealed the presence of myelofibrosis in fibrotic stage with pure red cell aplasia. Bone marrow aspiration resulted in a dry tap. Since the patient was totally dependent on red blood cell (RBC) transfusions, he was treated with prednisone (1 mg/kg/day) for one month, which raised his hemoglobin level to 10 g/dL. However, steroid therapy was discontinued because the patient developed diabetes mellitus and severe pneumonia, which was successfully treated with antibiotics. Two months after the discontinuation of the steroids, the patient was treated with hydroxyurea because his splenomegaly worsened (19 cm) and his platelet count increased to 1.080.000/µL. However, hydroxyurea was discontinued two weeks later because the patient developed anemia (Hb 7,1 g/dL), which was corrected with RBC transfusions. Since the patient became heavily dependent on RBC transfusions (8 packed RBC units per month), a second line immunosuppressive treatment with oral cyclosporine (CyA) (5 mg/kg/day) was started. At this moment, three months since the start of CyA therapy, the patient’s hemoglobin level and platelet count are stable at 9,1 g/dL and 310.000/µL. In addition, a CT scan revealed that there are no signs of relapse of the thymoma, one year after its surgical ablation. Conclusions. The pure red cell aplasia of our patient was most likely associated with idiopathic myelofibrosis, because he had completely recovered from the thymoma when it became manifest and is still in remission. Immunosuppressive therapy with CyA was successful in improving the hematologic parameters in our patient. This case supports the hypothesis that myelofibrosis with pure red cell aplasia is caused by autoimmune mechanisms.

References

mRNAs were increased in CD34+ of 8/10 evaluable patients (58-1013) vs 19 pg/mL. Compared to controls, baseline VEGF 331 pg/mL at baseline, were increased: TGF-β mean PPP levels of growth factors, in the 15 patients ing the first month of treatment. Compared to control, evaluable because the drop out occurred before achiev-

ing the first month of treatment. Compared to control, mean PPP levels of growth factors, in the 15 patients at baseline, were increased: TGF-β 5156 pg/mL (0-18951) vs 3019 pg/mL of the control; VEGF 331 pg/mL (58-1013) vs 19 pg/mL. Compared to controls, baseline mRNAs were increased in CD34+ of 8/10 evaluable patients for VEGF, in 8/10 for TGF-β but significative only in 4 cases. The KDR expression resulted extremely variable: of 8 evaluable patients, mRNA expression was not detectable in 4 and increased in 2 of them. Although experiments are still in progress, the available data so far seem to indicate a concordance between PCR and cytofluorimetric analysis. After thalidomide treatment, PPP levels of TGF-β augmented in all patients but significantly in 2 cases while PPP levels of VEGF followed an opposite progress but not in a significative way. Also, by semiquantitative RT-PCR, performed on CD34+, mRNA of TGF-β increased significatively in 3/5 (in 2 cases after three months) and decreased in 1/5, VEGF decreased in 2/5 and increased in 1/5 and KDR resulted significantly increased in 1/4 and highly decreased in 1/4 following treatment. These results confirm that angiogenesis is stimulated and may play a role in the pathogenesis of MMM. On the other hand, our data do not clearly support the hypothesis of an inhibition of angiogenesis by thalidomide.

PU316
REVERSION OF LOEFFLER’S ENDOCARDITIS (LE) BY IMATINIB IN EARLY STAGE CLONAL HYPEREOSINOPHILIC SYNDROME
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LE is a rare disease caused by endomyocardial fibrosis during hyper eosinophilic syndrome (HES). Congestive heart failure may result from alterations of both systolic and diastolic functions; mural thrombi may develop and originate to systemic emboli. Therapeutic options are often unsatisfactory, and regression of intracardiac deposits are seldom described. A 37 year-old man was referred to our unit in December 2002, because of hyper-eosinophilia and infiltrative cardiopathy recently discovered. Based on the suspicion of intraventricular thrombi raised by echocardiography, the patient had started anticoagulation with dicumarol. Basal work up showed marked eosinophilia (14000/mm³) with partially degranulated eosinophils and ring-hole nucleus in some of them, thrombocytopenia (54.000/mm³) and raised serum LDH (526 UI/mL). Bone marrow cytology showed eosinophil hyperplasia with signs of dysplasia. Cytogenetics performed on 24 h unstimulated culture showed 46 XY add 17q(25) in all 20 metaphases observed. Standard transthoracic Doppler echocardiography showed left ventricular eccentric hypertrophy and two-dimensional imaging in apical views revealed a large in plus image, completely involving the apical region, with apparently pedunculated corps floating in the LV chamber. Doppler analysis showed mild mitral and pulmonary valve regurgitations, normal diastolic pattern of both left and right ventricular filling. Twelve minutes after i.v. infusion of 5 ml of the myocardial contrast agent SonoVue (Bracco Spa, Milan, Italy) and its disappearance from the internal cavity with residual persistence only at the endo-myocardial level, the structure previously appearing as an intracardiac mass associated to floating formations appeared as a thick endo-myocardial infiltration involving also papillary muscles and tendinous chords, which simulated mobile thrombi at standard echography, well identifiable and synchronous with the phases of cardiac activity. Diagnosis was Eosinophilic leukemia (EL) with subendocardial infiltra-

tion. Imatinib 200 mg/d (provided for compassionate use by Novartis Farma Italia S.p.A) was started, while continuing dicumarol. After one month blood counts were normal (Hb 12.4 g/dL; WBC 5.500/mm³; eos. 90/mm³; plt. 215.000/mm³). Bone marrow eosinophils were 10% and cytogenetics showed a major cytogenetic response [10 metaphases 46 XY and two 46 XY add 17q(25)]. Echocardiographic evaluation repeated at this time showed drastic reduction of the subendocardic wall infiltration. LV mass index and relative diastolic wall thickness were both within the normal limits. After the SonoVue injection the infiltration was hardly detectable at the level of the LV myocardial walls. The infiltration of papillary muscles and tendinous chords was not yet detectable and the apical region showed only a minor involvement. The patient has now returned to his work, does not complain of dyspnoea and takes imatinib 100 mg/day. Using the innovative echocardiographic approach which exploits the capacity of contrast agents to visualize the cardiac walls, we demonstrated the non-thrombotic origin of the in plus endocardiac images in early stages LE and their rapid resolution following imatinib treatment.
INTERFERON TO TREAT ESSENTIAL THROMBOCYTHEMIA DURING PREGNANCY: 4 CASES AND A LITERATURE REVIEW

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ET in pregnancy is often complicated by abortion, and other peri-partum gynecologic problems may arise, most of them due to placental injuries secondary to thrombosis. We describe 9 pregnancies in 4 women affected by ET. Four pregnancies were carried out without alpha-interferon (IFN-α) therapy and exited in 2 intrauterine deaths, 1 spontaneous abortion and 1 neonatal death. The patients were treated with IFN-α during the other 5 pregnancies: 2 ended in preterm normal infant and 3 in full-term deliveries. We think that ET must be considered a risk factor for a pregnancy, and it is mandatory to treat a patient who already had complications during a previous pregnancy. IFN-α is increasingly been used during pregnancy, although pregnancy is still listed as a contraindication to IFN-α treatment. Antiproliferative drugs could impair fetus development causing abortion, congenital malformations or intrauterine growth restriction. ASA can be used, in order to reduce the ischaemic placental damage. It is reported to be effective in many vaso-occlusive manifestations, but its efficacy in reducing pregnancy complications is not proven. We analysed 31 report from the literature, listing a total of 263 pregnancies in 136 women affected by ET. Our cases and the published series suggest that fetal outcome is improved by therapy and that IFN-α may be the right option.

We have studied 28 ET patients treated with α-IFN M/F 12/16, median age 35 (range 19 - 54). Before starting α-IFN therapy in all patients thyroid autoimmunity was investigated by dosing in the serum anti-thyreoperoxidase-Ab (TPOAb) and anti-human-thyreoglobulin-Ab (HTGAb); thyroid function was evaluated by serum levels of FT4, FT3, TSH. Patients with thyroid dysfunctions at diagnosis did not receive IFN treatment. IFN therapy was well tolerated in all patients and in all the treatment was beneficial.

HYPERCYTOSIS AND SPLEEN VOLUME

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Patients submitted to long-lasting treatment by alpha-interferon (α-IFN) may develop autoimmune thyroid dysfunctions with an unknown physiopathological mechanism. Many reports have appeared describing cases of thyroid dysfunction in patients with chronic viral hepatitis while on α-IFN therapy, with an incidence varying from 2.5 to 45.3%.
showing: (a) erythrocytosis (Table 1), (b) thrombocytosis (Table 2), (c) reactive leukocytosis (Table 3), when spleen enlargement was mild or absent at physical examination.

Table 1. Main data of patients showing erythrocytosis.

<table>
<thead>
<tr>
<th>Pts</th>
<th>M/F</th>
<th>Hct% *</th>
<th>RCM* ml/kg</th>
<th>SV* ml</th>
<th>CC</th>
<th>Palpable splenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>69</td>
<td>50.1</td>
<td>48</td>
<td>39.9</td>
<td>180</td>
<td>13.1</td>
<td>6 Pts</td>
</tr>
</tbody>
</table>

*expressed as mean (range is in brackets); RCM: red cell mass; SV: spleen volume; CC: cranio caudal diameter.

Table 2. Main data of patients showing thrombocytosis and affected by ET, at diagnosis.

<table>
<thead>
<tr>
<th>Pts</th>
<th>M/F</th>
<th>Plts*</th>
<th>M</th>
<th>Plts*</th>
<th>F</th>
<th>SV*</th>
<th>CC</th>
<th>Palpable splenomegaly</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>24/44</td>
<td>704</td>
<td>700</td>
<td>299</td>
<td>10.5</td>
<td>35 Pts</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*expressed as mean (range is in brackets); Pts: patients; Plts: platelets; SV: spleen volume; CC: cranio caudal diameter.

Table 3. Main data of patients showing leukocytosis.

<table>
<thead>
<tr>
<th>Pts</th>
<th>Cause of leukocytosis</th>
<th>WBC* ×10^3/µL</th>
<th>SV* mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Sepsis (2), Abscess(1), Gut perforation(2)</td>
<td>45 (35-55)</td>
<td>160 (150-166)</td>
</tr>
<tr>
<td>13</td>
<td>G-CSF stimulation for allo BMT</td>
<td>50 (30-80)</td>
<td>470 (350-1200)</td>
</tr>
<tr>
<td>22</td>
<td>G-CSF stimulation for aut BMT</td>
<td>10.4 (8-30)</td>
<td>365 (60-920)</td>
</tr>
</tbody>
</table>

*expressed as mean (range is in brackets); Pts: patient; SV: spleen volume; WBC: white blood cells.

All patients with possible other causes of splenomegaly (such as portal hypertension, HCV positivity, thalassemia or increased bone marrow fibrosis) were not considered. In all patients SV was detected by ultrasonography (US); in 32 patients both US and CT were used. The correlation between US and CT was excellent; US seems to overestimate the volume of 15-20% compared with the CT scan. In a majority of cases elevated red cell mass (RCM) and thrombocytosis did not modify SV. Reactive leukocytosis either did not affect SV or induced minimal changes. Growth factor induced leukocytosis caused constant SV increase. Although splenic parenchyma is the main trapping filter for circulating cells, increased blood count do not cause constantly SV enlargement. Diseases with elevated peripheral blood count and splenomegaly may have mechanisms of spleen enlargement other than circulating cell trapping, probably extramedullary hematopoiesis.

Spleen enlargement is an important finding in several diseases and the palpable measurement alone is unreliable; spleen can be enlarged although non palpable, and sometimes slightly palpable but not truly enlarged. The use of an imaging technique is then recommended to obtain an accurate and objective volume measurement. Although ultrasonography (US) is the preferred modality for the assessment of spleen volume, its accuracy has not been inquired extensively in the literature: few papers are available about its accuracy, and its repeatability is considered poor. Computed tomography is very accurate and the data so obtained permit to exactly define the profile of the spleen, free from shape variability. The voxel count method has been demonstrated to be accurate and repeatable. We studied thirty-two patients affected by hematologic pathologies: myeloproliferative diseases (18 ET, 3 MMM, 3 PV), reactive leukocytosis (2 sepsis and 1 abscess) and growth factor induced leukocytosis (3), NLH (1), HCL (1). For CT scan, the spleen was completely included in a single acquisition, and the data sent to two different workstations for volume measurement. To assess interobserver reproducibility all measurements were done twice by the same operator. To avoid bias, the repeated measurement were performed at intervals longer than 60 days and the operator was blinded to the previous result. For interobserver reproducibility, another operator, unaware of the previous results, repeated the volume measurement in 21 patients. For ultrasound evaluation, the same operator performed all spleen US scans using a 2.5/3.5-M Hz broadband curvilinear probe. Perimeter, longitudinal diameter and area, defined as the maximum measurements with splenic borders and angles clearly defined, were measured, and volume (in milliliters) was calculated automatically. For each subject, the mean value of three measurements repeated on the same occasion.
was calculated and recorded for final analysis. To assess the intraobserver reproducibility twelve unselected patients were studied by repeated US measurements at one week interval by the same operator while for the interobserver reproducibility another operator, unaware of the previous results, repeated the US scans. Both CT and US evaluations were performed in fasted patients. Statistical evaluation, including testing, analysis of variance with Bonferroni correction and Pearson correlation, were performed with SPSS for Windows software (version 9.0; SPSS, Chicago, IL, USA). Interobserver concordance of the CT based volume assessment, calculated in 21 spleens, was excellent: the two-tailed P value was 0.0018, highly significant, as was the coefficient of correlation (r=0.9996; one tailed p<0.0001). Intraobserver reproducibility in the delineation of the spleen for CT scans was excellent, too, with a correlation coefficient (r) of 0.9987 and one tailed p value <0.0001. Both intraobserver and interobserver reproducibility of measurements of spleen volume by US was excellent, with Pearson values of 0.999 and 0.997, respectively. Spleen-volume measurements by US and by CT scanning were strongly correlated, with a Pearson value of 0.945 and a p value of correlation highly significant (<0.0001). Although CT scans resulted to be more accurate, our study showed that US measurement volume is sufficiently accurate and repeatable for clinical use in the staging and follow up of several hematologic disease.

PU321
HYDROXYUREA IN PRIMARY THROMBOCYTOSIS: CASE-CONTROL STUDY ON DRUG TOXICITY IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS AND A VERY LONG FOLLOW-UP
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Background: Hydroxyurea (HU) is the most widely used drug to reduce platelet count in patients with Essential Thrombocythemia (ET) and polycythemia vera (PV). However, HU may be ineffective, or may induce undesired collateral effects; in particular it may favour the evolution into acute leukemia (AL) and/or myelofibrosis (MF) or the occurrence of cancer. Patients: We report the data of 36 MPD patients (13 males, 23 females, 19 PV, 17 ET, mean age 54.2±14.35 years), all followed in a single Center, who receive HU (mean maintenance dose 5 g/week) for at least one of the following causes: 1) age over 60 years 2) previous major thrombotic event 3) platelet count over 1500×10^12/L. Twelve out of the 19 patients with PV underwent phlebotomies. The controls are 36 patients matched for sex, age and diagnosis but without HU therapy. 11 PV control patients underwent regular phlebotomies. In both groups aspirin 100 mg/day was used in the presence of microvascular disturbances, cardiovascular risk factors or previous thrombosis (25 patients and 20 controls); the patients with venous thrombosis received warfarin (5 patients and 5 controls). Methods: The analysis of variance test was used to compare means for statistical significance; the rate of complications in patients and controls were compared by the χ² test with the Yates correction (p<0.05 was considered significant) and the Kaplan Meier curve method to estimate the probability of overall survival as function of time. Results: The total median follow up was 11.92 and 7.38 years respectively in patients and controls. The median follow up with HU in patients group was 5.46 years In 26 out of 36 patients (72%), RBC macrocytosis was observed in a median time of 0.85 years (range 2 months-15 years); 14 patients (38.8%) stopped HU: 6 (16%) due to lack of efficacy, 1 for unacceptable macrocytosis, 1 because leg ulcers and 6 for achieved control of platelet count. At present: 12 patients (3 cancers, 2 acute leukemias, 2 thrombosis, 2 hemorrhage and 3 other causes) and 7 controls (2 cancers, 1 thrombosis, 1 hemorrhage and 3 other causes) died. Comparing the follow-up after the start of HU therapy and the total controls follow up, no statistical differences was observed in thrombotic, hemorrhagic and overall survival. Conclusions: The use of HU in ET and/or PV and high thrombotic risk seems not to reduce the overall survival nor to induce an higher incidence of AL and cancers. In contrast, HU reduces the incidence of thrombosis.

PU322
PORTAL THROMBOSIS IN POLYCYTHEMIA VERA (PV) AND ESSENTIAL THROMBOCYTHEMIA. OBSERVATION STUDY OF A LARGE COHORT OF PATIENTS WITH A VERY LONG FOLLOW-UP
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Background. Thromboembolic and hemorrhagic events are frequently encountered in the course of PV and ET, being a major cause of morbidity and mortality. While venous thrombosis mainly involve deep veins of the legs, splanchnic thrombosis even occur in ET and PV, even in a latent form or at an early clinical stage. Several studies are available on Budd-Chiari syndrome, but little is known about portal vein thrombosis (PVT) in such patients. Patients. 32 cases of PVT in PV/ET (11 PV, M = 7, F = 4; 21 ET, M = 4, F = 17) diagnosed according to the Polycythemia Vera Study Group criteria are reported. Mean age at diagnosis of PV/ET
PU323
EOSINOPHILIC DISORDERS AND HYPEREOSINOPHILIC SYNDROMES
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Eosinophilic disorders (ED) include idiopathic hypereosinophilic syndrome (HES), familiar hypereosinophilia, chronic eosinophilic leukemia and myeloproliferative disorders with eosinophilic leukemia. Patients with HES are defined based on peripheral blood eosinophilia >1500/mL lasting for at least six months without recognized secondary cause, marrow blasts <5%, absence of dysplastic changes in the non-eosinophilic lineages. Treatment has been generally initiated according to symptoms and/or relevance of organ infiltration since the mostly used drugs, hydroxyurea and α-interferon, are capable to control eosinophil proliferation (in about 50% of cases) and ameliorating symptoms. Clinical response, however, is short lasting and disease recurrence occurs in the majority of the cases. Imatinib mesylate, a small molecule that inhibits tyrosine kinase oncogenes including bcr-abl and receptors for stem cell factor (c-Kit) and platelet-derived growth factor (PDGFR), has been recently reported to be active also in HES. In this disease a novel tyrosine kinase created by the fusion of the PDGFR-α and the FIP1L1 genes targeted the imatinib molecule. We report here on twelve ED patients observed at our institution.6/12 are at this time followed (median time 12mo, range 1-96) without treatment (group A) because of asymptomatic disease or minimal organ infiltration. In this group the median age is 63 y (range 56-71), median eosinophil absolute count is 1.550/mL (range 1.500-2.000). The remaining 6 patients (group B) suffered from symptoms showing, in addition, organ infiltration with functional impairment. The median time of diagnosis in this group is 3 mo, range 1-7mo, the median age is 52 y (range 23-79) and the median absolute eosinophils count is 2.670/mL (range 1.900-8.815). In the group B 3 pts have been treated with steroids (median time of 7mo, range 6-72) at maintenance dosage of 0.5 mg/kg and 1 pt have been treated with steroids at same dose and hydroxyurea 1gr/three times a week. We start treatment with imatinib in 2 pts of group B: the first is an untreated pt eligible to therapy; the second stopped treatment with hydroxyurea and steroids because of severe side effects. Patients will receive 100 mg of imatinib once daily increasing at 200 mg/day plus steroids after two weeks and at 400 mg/day after four weeks (maximum dose) until response. We will report the results of our study to evaluate the correlations of biological features of HES with clinical response to imatinib and to determine safety and efficacy of this agent in this setting.

PU324
CHRONIC MYELOID LEUKEMIAS
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Survivin (SVV) is an inhibitory protein of apoptosis overexpressed in various human cancers and not detectable in normal differentiated tissues. It has been reported that patients whose tumours expressed SVV had a decreased overall survival, an increased rate of recurrence and resistance to therapy. Since SVV could be a promising target for new anticancer interventions, we evaluated its expression in 44 patients with typical Ph-positive CML (24 males and 20 females; 30 pts in CP, 14 pts in A/B phase; all treated with either HU or α-IFN or STI-571). After patients' informed consent, peripheral granuloblastic cells, were isolated by density centrifugation, total RNA was extracted and SVV expression was detected by using a quantitative RT-PCR. Venous blood samples collected from 20 healthy people served as controls. We found high, although variable, SVV expression in all CML samples (mean 3.319±8.586±8.52 log/ratio) whereas SVV levels were very low or absent in healthy controls (mean 1.66±2.36
log/ratio) (p<0.0001). A cut-off value of 15.52 log/ratio was chosen to classify CML samples as SVV-positive (40 patients) and SVV-negative (4 pts.) cases. Within SVV-positive patients, no differences in SVV levels were detected among the different phases of the disease (p=0.1), neither in regard with patients’ older age (p=0.3), Hb concentration (p=0.3), PLT-count (p=0.1), hematopoiesis (p=0.2). A trend toward an increase in SVV expression was observed in those patients with a WBC-count >20,000 mm\(^3\) (p=0.05). By contrast a significant reduction in SVV expression was observed in those patients undergoing treatment with α-IFN and STI-571 as compared to those on HU-therapy (p=0.02). SVV levels correlated with Ph-chromosome positive cells (p=0.01), with WBC-count (p=0.001) and LDH serum levels (p=0.003) but not with Hb concentration (p=0.3) and PLT-count (p=0.6). Our results suggest that SVV analysis in CML cells could become not only a useful laboratory and clinical marker but also an attractive candidate for devising new targeted therapies.

**PU325**

CML PATIENTS WITH COMPLETE KARYOTYPIC RESPONSE TO STI571 (GLIVEC) THERAPY SHOW RAPID REDUCTION OF BCR-ABL TRANSCRIPTS

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Introduction. Chronic Myelogenous Leukemia (CML) is characterized by a bcr-abl gene that encodes a fusion protein with deregulated tyrosine kinase activity inducing tyrosine phosphorylation and alteration of several signaling pathways that deregulate cellular growth and prevent apoptosis. STI571 (Glivec-Imatinib mesilate), a BCR-ABL tyrosine kinase inhibitor, inhibits the growth, induces apoptosis, and is a promising drug entered into clinical trials as a candidate treatment of BCR-ABL positive CML. The Italian Cooperative Study Group on CML (ICGS on CML) has activated a phase II multicenter study (CML002/STI571) to evaluate efficacy and safety of Glivec in patients with Ph+ chronic myeloid leukemia, in chronic phase failing IFNα for resistance or intolerance. The endpoints of this study were: cytogenetic response, molecular response, time to progression, and overall survival. Conclusions. 1. The amount of the BCR/ABL transcript before treatment does not predict for the (cytogenetic) response to treatment, at least in this population of late chronic phase patients. This might not apply to previously untreated, early chronic phase patients. 2. A significant and substantial decrease of the transcript was shown during the first half year. During the subsequent 6 months no further decreased was detectable. To understand whether molecular response will level off or will improve with time, more samples and a longer treatment time are required. 3. Assessing the frequency of complete molecular response requires: a. more time and more data on early chronic phase patients; b. a definition of complete molecular response, which is still lacking. 4. At any time point in the great majority of cases the BCR/ABL transcript level was significantly lower in peripheral blood than in bone marrow samples. To assess the kinetics of the response, peripheral blood cells are valuable, but to assess the degree of the response, bone marrow samples are likely to provide a more precise estimate.

Funding: this work was supported by grants from MURST COFIN 2001 and 2002, A.I.R.C., ATENO60% target projects, “Hairshow” A.I.L., “Fondazione del Monte di Bologna e Ravenna”, Fondazione Carisbo.

The enzyme protein farnesyltransferase has emerged as an important target for the development of anticancer agents. To date, specific farnesyltransferase inhibitors (FTIs) have been synthesized and represent a new class of chemotherapeutic agents that are target signal transcription pathways responsible for the proliferation and survival of diverse malignant cell types, without toxicity to normal cells. At moment, at least five different FTIs: SCH66336, R115777, FTI 277, L744832 and BM5214662 are in clinical trials worldwide. A few data are now available to evaluate the in vitro effects of FTIs in chronic myeloid leukemia. Indeed, the current study represent an initial attempt to test, in vitro, the sensitivity of M2 and M1 Ph+ CLL cell lines to FTIs. In particular we tested the non peptidomimetic FTI R115777 (Janssen-Cilag) and FTI SCH66336 (Schering-Plough), and peptidomimetic inhibitor FTI277 (Calbiochem) and L744832 (Biomol). Cell cycle analysis of two cell lines, after treatment for 48h, with different concentration (0.5 mM; 1 mM; 10 mM; 20 mM)
PU327

PROLONGED GLIVEC TREATMENT IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS AND PERIPHERAL BLOOD STEM CELLS HARVEST


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The use of high-dose chemotherapy followed by autologous PBSC transplantation is a controversial issue in CML patients. Glivec is considered the treatment of choice for patients not eligible for allogeneic transplantation. Glivec induces 70-80% of cytogenetic and morphologic complete remissions (CR), but with a low rate of molecular CR. When relapse occurs after or during STI treatment there are no standard guidelines regarding the best therapeutic strategy. Autologous transplantation could be considered a possible salvage therapy in patients relapsed and not suitable for allogenic transplantation. There are no data regarding feasibility, when and how to collect PBSC during Glivec treatment. We evaluated PBSC collections in 3 patients positive for t(9;22) at diagnosis and positive for bcr-abl at 210 rearrangement in qualitative PCR (BIO-MED 2 procedure, sensitivity 10(-6)). Frontline therapy was hydroxyurea followed by Glivec at 400 mg/day dose. Two patients achieved a complete cytogenetic response after 5 and 7 months, but they never achieved molecular remission. One patient achieved cytogenetic and molecular remission after 3 months. Following major cytogenetic response, patients received 4g/m2 Cyclophosphamide and G-CSF (5µg/kg) for PBSC collection by apheresis. Glivec was not discontinued during PBSC mobilization and collection. PBSC collection required a median of 2 apheretic procedures (range 1-3). The CD34+ cell collections were 2.7 and 6.0×10^6/kg, and the patient in molecular complete remission collected 9.0×10^6/kg. All PBSC collections were bcr-abl negative in qualitative PCR. When CML patients in chronic phase, are treated with Glivec and CTX plus G-CSF, mobilization is associated with the harvest of a quantitatively adequate collection of bcr-abl negative PBSC. Our experience seems to confirm that among patients receiving Glivec for CML chronic phase, the mobilization with CTX plus G-CSF is associated with a quantitatively adequate collection of bcr-abl negative PBSC. This strategy could be useful as a rescue for patients relapsed during Glivec treatment.

PU328

COMBINATION OF FLUDARABINE, CYTARABINE AND ANTHRACYCLINE±IMATINIB-MESYLATE AS SALVAGE THERAPY OF PATIENTS WITH PH+ ACUTE LYMPHOBLASTIC LEUKEMIA AND CHRONIC MYELOID LEUKEMIA IN ACCELERATED OR BLASTIC PHASE


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Background and Aims. Fludarabine combinations with Ara-C and anthracycline result in a well-known synergistic enhancement of antileukemic activity and have been favourably employed in high risk acute leukemia and in advanced phase chronic myeloid leukemia. In the last years imatinib-mesylate has been shown to improve outcome of patients with chronic myeloproliferative and acute lymphoproliferative syndromes bearing t (9;22). Herein we report in this poor risk subgroup of Philadelphia chromosome-positive (Ph+) leukemia’s the preliminary results of a salvage therapy consisting of fludarabine, cytarabine and imatinib-mesylate. The rationale of this sequential treatment was first to produce a profound hypoplasia with fludarabine association and then favour the expansion of normal hemopoiesis by inhibiting with imatinib the Ph+ one. Patients’ feature and results. Nine patients, 4 with ALL (3 refractory to previous treatment, 1 relapsed following allogeneic bone marrow transplant), 5 with chronic myeloid leukemia, in accelerated phase (1), in
myeloid (3) or lymphoid (1) blastic transformation, were included at now. Median patients’ features were the following: age was 50 years (range 34–75), performance status according to WHO was 2 (1–3), patients had received 3 prior regimens (range 0–4), in CML patients the chronic phase had a duration of 86 months (72–100), at start of therapy leucocytes, Hb and Plt were 17×10⁹/L (72–100), 9.9 g/dL (6.5–12.7) and 139×10⁹/L (6–1118), respectively. Four patients were given FLAN (Fludarabine, Ara-C and mitoxantrone), 5 patients were administered FLAD (Fludarabine, Ara-C and liposomal daunorubicin). Three out of 4 ALL patients achieved a complete hematologic response (75%); among CML patients, 2 (the patient in accelerated phase and 1 in myeloid transformation) returned in a second chronic phase (40%), 3 did not respond (60%). Six patients went on with 600 mg/day imatinib-mesylate therapy at a median interval of 1,5 month (1–5) from the start of chemotherapy. All the patients were hematologically responsive to imatinib without significant hematologic toxicity. Four patients reached a complete cytogenetic response (the CML in the accelerated phase and 3 ALL patients). Three patients (with ALL) underwent allogeneic bone marrow transplantation. The responsive ALL patients relapsed after a median of 7 months (range 4–11). Among CML patients, one (treated in myeloid blast crisis) relapsed after 3 months, the other, treated in accelerated phase, still maintains hematological and cytogenetic complete response at 30 months from the start of therapy. Three patients are still alive, 6 have died. The median survival is 14 months (range 2–30). Conclusions. In the present poor risk series of patients with Philadelphia chromosome–positive leukemias the sequential administration of an AML chemotherapy and imatinib mesylate proved to be a feasible and effective salvage therapy.

PU329
THE TRANSITORY OCCURRENCE OF A +8 CLONE DURING COMPLETE CYTOGENETIC RESPONSE AFTER IMATINIB THERAPY FOR A PHI+CML IN BLASTIC CRISIS
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Additional chromosome abnormalities (clonal evolution-CE-), particularly trisomy 8, duplication of the Philadelphia chromosome (Ph) and iso (17q) have been traditionally associated with evolution to accelerated or blastic phase in patients (pts) with chronic myeloid leukemia (CML). In the recent years, the use of Imatinib lead to an impressive occurrence of cytogenetic responses (CRs), particularly complete CRs (CCR). Similarly to traditionally treated pts, CE occur either in Ph positive (Ph+) or in Ph negative (Ph-) cells and this fact has been associated with unfavourable prognosis. We describe here the case of a Ph+ CML pt who developed a transitory hyperdiploid +8 clone during hematologic and cytogenetic CR after Imatinib therapy for a very precocious myeloid blast crisis. A Ph+ CML (Sokal 0.608) was diagnosed in a 35 years-old nurse on January 2002. α IFN 2b therapy was started up to a maximum tolerated dose of 5–10 MU/day. On April 2002, a myeloid blast crisis (BC) was diagnosed. Karyotype showed a 46,XX,t(9;22)(q34;q11) with no additional abnormality in all the 20 metaphases observed. Grade II splenomegaly was present. Therapy with Imatinib was started (600 mg/d) with immediate hematologic toxicity. Imatinib was discontinued and successively administered again at lower doses. On June 20, 2002 a CR was demonstrated. On August 2002 all the 20 metaphases analysed were Ph−, but 7 of them (35%) carried a +8 karyotype. On November 11,02, 4 out of 20 Ph1- metaphases carried with trisomy 8. On February 2003 the karyotype was 46,XX, with no additional chromosome change. The patient has been proposed for a VUD transplant. Fifteen months after BC evolution and 14 months after starting Imatinib therapy she is in complete hematologic and cytogenetic remission. The occurrence of additional cytogenetic abnormalities is usually an indicator of poor prognosis in conventionally-treated Ph1+ CML patients. It is still not clear the real extent to which such abnormalities occur and whether this fact may affect prognosis in Imatinib treated ones. In a recent study the incidence was shown comparable to that of α IFN treated pts (15%) and a stright correlation with a poor outcome was reported. In our patient, two distinct elements are worth of note: 1) the occurrence of CE in a myeloid BC during a long lasting CCR due to Imatinib therapy and 2) its transitoriness. The fact that BC occurred very early (2 months after diagnosis in a 0.608 Sokal score patient), may have had a favourable effect on the subsequent course of the disease, by allowing the persistence of a high number of residual viable normal stem cells in the bone marrow.

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References
PU330
CYTOGENETIC RESPONSE TO IMATINIB MESYLATE TREATMENT IN CHRONIC MYELOID LEUKEMIA

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Imatinib-Mesylate (STI-571) has been demonstrated to induce durable major cytogenetic response in approximately 80% of CML patients. Only a minority of patients achieve a molecular remission at RT-PCR and quantitative-PCR analysis. Here we analyse 21 patients (15 M, 16 F, median age 50 y) affected by CML, diagnosed between 1991 and 2002 and treated with conventional dose Imatinib-Mesylate from August 2000. Sokal score was calculated only in 12 patients and was low, intermediate and high in 5, 4, 3 patients respectively. Only 2 patients received Imatinib-Mesylate as first line therapy, the other 19 were initially treated with Interferon only, Interferon and cytarabine, hydroxyurea, busulfan, 6-mercaptopurine, autologous bone marrow transplantation or other chemotherapy regimens. None of the 21 patients were eligible for related sibling BMT. When Imatinib-Mesylate treatment has been started 18 patients were in chronic phase and 1 patient in accelerate phase, whereas 2 patients were in blast evolution. They had been treated with Imatinib for a median of 16 months. In 17/21 patients cytogenetic analysis was available after a median of 6 months of therapy: complete cytogenetic response (Ph+ 0%) was obtained in 7 patients (41%), partial cytogenetic response (Ph+: 1-35%) in 4 patients (23.5%), minor cytogenetic response (Ph+: 35-95%) in 2 patients (11.7%) and no response in 4 patients (23.5%). Evaluation after a median of 12.5 and 24 months confirmed the previous results. No molecular response at PCR analysis was demonstrated in these patients. The rate of complete hematologic response was 94% and the rate of major cytogenetic response was 64.7% after a median of 6 months of therapy. Six out of 21 patients presented mild-moderate adverse events related to Imatinib-Mesylate administration: thrombocytopения, flu-like syndrome, hepatic alteration, eritematous rash, thrombosis, fever, peri-orbital edema and pericarditis, but none patients discontinued treatment definitively. Nineteen (90.4%) patients are alive with a median follow-up of 14 months from the beginning of Imatinib; 2 patients who were treated in blastic evolution died after 9 and 23 months of therapy respectively. Our data confirm the favourable impact of Imatinib-Mesylate in the treatment of CML. A longer follow-up needs for a better definition of overall survival of CML patients in Imatinib era.

PU331
APPEARANCE OF TRISOMY 8 IN PHILADELPHIA CHROMOSOME (PH)-NEGATIVE CLONE DURING IMATINIB MESYLATE THERAPY

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During imatinib mesylate (STI571) therapy in chronic myeloid leukemia (CML) the appearance of chromosomal abnormalities in Ph-negative clones is described. We report about a case of appearance of trisomy 8 in Ph-negative clone during imatinib mesylate therapy. In June 1999 a 76-year-old woman was diagnosed with Ph-positive CML chronic phase. She received hydroxyurea (HU) orally with a good hematological response. Because of development of accelerated phase of CML not responding to increased HU dose, in April 2002 the patient started to receive Imatinib 600 mg/day with normalization of leukocyte count and differential WBC count, but a mild anemia persisted. After six months, bone marrow aspirate showed a reduction of the hyperplasia of granulo and megakaryopoiesis, but also a presence of signs of dysplasia of granulopoiesis and erithropoiesis. The karyotype analysis showed the contemporary presence of two cellular lines: a) 46, XX, and b) 47, XX, +8, one Ph-negative metaphase. Imatinib administration was continued. The morphological pattern of bone marrow six month later was substantially resembling to the previous one. The karyotype analysis showed the persistence of a Ph negative clone with +8, no Ph+ metaphases were observed. Discussion and conclusions: appearance of abnormal Ph-negative clones was already reported in CML patients during αIFN/convventional cytostatic agents treatment and recently in CML patients during STI571 treatment. The development of chromosomal aberrations in Ph− clones after STI571 therapy is a new phenomenon, to be further clarified. It is under discussion whether the Ph− clone could be considered an evolution of the disease, or a de novo disease, or a sign of appearance of therapy-related myelodysplasia or acute leukemia; it also could be related to previous duration of disease or prior therapy. Moreover it would be relevant to understand if +8 could represent a finding of particular evidence in a clinical-prognostical view: a longer number of patients is needed with a long-term follow up. These data suggest that during STI571 therapy is recommendable to perform conventional cytogenetics at regular intervals and to assess the morphology of the bone marrow aspirate.

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The chronic myeloid leukemia is a clonal myeloproliferative disorder of pluripotent stem cells, characterized by clinical course biphasic or triphasic; by chromosome Philadelphia t(9;22)(q34;q11) and a chimeric BCR/ABL gene. There are three forms of BCR/ABL fusion gene, involving ABL exon 2 and including different exons of BCR. Transcripts b2a2 and b3a2 code for the p210 protein; e1a2 for the p190 protein; c3a2 or e19a2 code for the p230 protein. Various rearrangements determine various leukemic phenotypes and different clinical courses. The p230 mRNA presence and p230 protein may predict an indolent course (N-CML). This milder phenotype, in patients with e19a2 transcript and without additional cytogenetic abnormalities is probably due to a p230 BCR/ABL mRNA and p230 protein low levels. In December 2000, we have observed a patient, 49 years old, male, affected by CML with cytogenetic abnormalities which presence of metaphases with a number from one to eight copies of isochromosome Ph1 and molecular finding (RT-PCR) p230 e19a2 BCR/ABL mRNA and p230 protein. The bone marrow aspiration showed Blasts+Promyelocites = 8%; the bone marrow biopsy showed hypercellular myeloproliferosis; Hb: 12.5 g/dL; GB: 38.3×10⁹/mm³; Plt: 58×10⁹/mm³; spleen: 2 cm; no blasts in pheipheral blood; Sokal’s Index: 0.75; WHO: 0. In January 2001 the patient started a therapy with IFN-α at 9 MU/daily. After one month we have observed the appearance of necrotic material. He started IFN-α and Scintigraphic study). The histological study showed osteolytic lesions at pubis and at right inferior limb (Rx 588×10³/mm³; spleen: 2 cm; no blasts in pheripheral blood; Sokal’s Index: 0.75; WHO: 0. In January 2001 the patient started a therapy with IFN-α at 9 MU/daily. After one month we have observed the appearance of osteolytic lesions at pubis and at right inferior limb (Rx and Scintigraphic study). The histological study showed necrotic material. He started IFN-α 5 MU daily and ARA-C 20 mg/m²/daily×10 courses/month. After three months, osteolytic lesions disappeared. In July 2001, the patient present rapid blast evolution: Hb: 6.8 g/dL; GB: 8.2×10³/mm³; Plt: 38×10⁹/mm³; blasts in peripheral blood: 1%; BM:A: blast crisis (M1) with blasts + promyelocytes 36%; BM B: myelogenous blast crisis. No additional abnormalities at cytogenetical investigation; no presence of alternative splincings and coexpressions. He showed resistance at STI571 and blast crisis conventional therapy. He died in September 2001. Unfortunately RT-PCR quantification could not be performed. It is possible to hypotize that the great number of copies of isochromosomes Ph1 in this case means that mRNA copies of p230 and p230 protein were present at a higher level, so explaining the not usual blast crisis in neutrophilic chronic myeloid leukemia.

Dendritic cells (DC) are potent antigen-presenting cells, derived from hemopoietic progenitors, able to promote specific T-cytotoxic immune reactions against a variety of antigens. Two populations of circulating DC have been described in adults: the myeloid CD11c+/CD123− subset (DC1) and the lymphoid CD11c+/CD123+ hi (DC2) one. The ability to recognize accurately and enumerate DC subsets represents the first step for understanding their role in the pathogenesis of a variety of human diseases, especially of neoplastic origin. A number of DC defects have recently been reported in both solid and hematological malignancies, such as chronic myeloid leukemia (CML), where DC have also been suggested to correlate to the response to interferon (IFN) treatment (Leukemia, 16:1484,2002). However, DC circulate in very small number in the blood and an accurate discrimination of the two subsets could result difficult to achieve with the standard cytofluorimetric procedures. This study aims to investigate the two DC subsets distribution in peripheral blood of 50 CML patients in chronic phase, previously treated with either α-IFN (n.15) or Gleevec (n. 35) and in 21 healthy control subjects by using a simple, rapid and accurate four-color cytofluorimetric method. A two laser flow-cytometer (Facsscalibur, Becton-Dickinson, S, José, CA, USA) and the following directly conjugated MoAbs have been used to analyse whole blood of both CML patients and controls: Lineage Cocktail (lin 1) FITC (CD3, CD14, CD16, CD19, CD20 and CD56), CD123 (anti IL-3) PE, anti-HLA-DR and low levels of lineage markers for monocytes, lymphocytes, and NK cells (lin 1 negative); DC2 cells were CD123+, HLADR+, line 1 negative and CD11c−, whereas basophilic granulocytes, which were also line 1 negative and CD123+, lacked HLADR expression. DC1 cells were identified as CD123+, HLADR+, line 1 negative and CD11c−. A marked and highly significant reduction was found in all CML patients of either percentage or absolute number of DC1-cells with respect to normal controls (mean% 0.08±0.07 vs 0.17±0.07; p<0.0001). DC2-cells, which are the natural IFN-producing cells, were similarly found deeply and significantly reduced in all patients (mean% 0.08±0.08 vs 0.12±0.05; p<0.0069). No difference in DC subsets was observed between patients undergoing IFN or Gleevec treatment and no significant correlation could be found with either WBC...
Morphologic changes, ultimately resulting in cell shrinkage, mitochondrial breakdown and DNA fragmentation, proceed from the cleavage and activation of caspase 7 and of downstream effector caspases 3 and 9 by activated caspase 8. Imatinib further promotes apoptotic cell death by relocating Bcl2 and BclXL to the cytosol, where they complex with activate caspase 8 and undergo a significant decrease.

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Imatinib circumvents the resistance to apoptotic death of clonal hematopoietic progenitors of chronic myeloid leukemia (CML) through multiple mechanisms, including the downmodulation of anti-apoptotic proteins, Bcl2 and BclXL, and functional restoration of pro-apoptotic Bad. Here we provide evidence for the involvement of Bid in the drug-induced death fate of CML cells. Among the BH3-only pro-apoptotic members of the Bcl2 family, Bid appears unique as it interconnects the extrinsic pathway initiated by death receptors to the intrinsic pathway of mitochondria-based cell death. Following death receptor signaling, caspase 8 is activated and cleaves the inactive p22 Bid conformer within an unstructured loop which exposes a N-myristoylated glycine. The resulting p7/myr-p15 Bid complex targets mitochondria with increased efficiency to trigger cell death. Furthermore, Bid establishes a pro-apoptotic cascade in which the BH3 domain of its 15 activated form induces Bax and Bak allosteric activation, including their homo-oligomerization at the mitochondrial intermembrane space for DC detection facilitates further studies exploring the distribution of DC subsets in other compartments, such as the bone marrow of CML patients.
Apoptosis represents an evolutionary conserved and tightly regulated form of death to which cells deliberately succumb. It plays a key role in the development of multicellular organisms and in the maintenance of homeostasis within the single organs. It removes, in fact, errant and potentially dangerous cells, including self-reactive lymphocytes, virus-infected and DNA-damaged cells. Accordingly, its abrogation contributes to the pathogenesis and progression of many diseases including cancer. The p210 bcr-abl tyrosine kinase, besides its effects on proliferation, prolongs the life expectancy of clonal myeloid progenitors by inhibiting apoptosis through multiple pathways including Bcl2 and Bcl-xL upmodulation, Bad phosphorylation and Bax translocation to the mitochondria membranes. Here we show that Imatinib, besides revoking all above anti-apoptotic signals, upregulates the death pathway proceeding from Fas receptor (Fas-R) and Fas ligand (Fas-L). Fas-R is a member of the tumor necrosis factor (TNF)/nerve growth factor receptor superfamily. It may be converted into a soluble form by the activity of metalloproteases. Fas-L ability of inducing apoptosis likely proceeds from Fas receptor (Fas-R) and Fas ligand (Fas-L). Fas-R is a member of the tumor necrosis factor (TNF)/nerve growth factor receptor superfamily. It may be converted into a soluble form by the activity of metalloproteases. Fas-L ability of inducing apoptosis likely results from its synergy with the membrane-bound form (DR4/DR5 ligand) in combination with Imatinib in the treatment of CML patients.

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IMATINIB MESYLATE (GLIVEC) CAN INDUCE HEMOLYTIC ANEMIA IN PU338 HETEROZYGOTES FOR GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY; 2 CASE REPORTS

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is one of the most common inherited X-linked disorders in humans. G6PD is expressed in all tissues, where it catalyzes the first step in the pentose phosphate pathway. Although most affected individuals are asymptomatic, there is a risk of acute hemolytic anemia triggered by infection and the ingestion of certain drugs and broad beans (favaism). We describe two female patients, diagnosed as having chronic myeloid leukemia (CML) and G6PD deficiency together with β-thalassemia trait in whom Glivec treatment induced a chronic nonspherocytic hemolytic anemia. Two 72- and 63-year-old females presented in November 2001 and July 2001 with clinico-hematologic findings consistent with CML in early chronic phase. Cytogenetic analysis and RT-PCR revealed the classical t(9;22) and p210 expression with b3a2 transcripts. At onset hemoglobin (Hb) was 11.8 and 12.3 g/dL, M CV 64 and 75 fL, with normal ferritin serum levels. Both patients had β-thalassemia trait. Sokal risk score was intermediate for case #1 and low for #2. Hydroxyurea reduced WBC count and treatment with an oral form of Cytarabine Octofosfate - YNK01 and alfaIFN was started. This scheduled regimen was interrupted after 2 and 5 months because of mood alteration in case #1 and intolerance in case #2. No hematologic toxicity more than grade 2 WHO was seen during IFN-ARA-C treatment and particularly no signs of anemia were ever found. Both patients were then shifted to Glivec treatment at standard dose of 400 mg/day. At this time the patients were in complete hematologic response and the patient #1 was in minor cytogenic response at the 6 month control. After starting Glivec both patients showed progressive Hb reduction with LDH, reticulocytes, total/unconjugated bilirubin increases, low haptoglobin and negative Coombs’ test without any symptom of acute hemolytic event. Because of anamnestic evidence of a father and a son hemizygotes for G6PD deficiency, G6PD activity was tested and found reduced to 28% and 82%, respectively. Despite intermittent administration of Glivec, r-EPO and folic acid treatment, patient #1 required red cells transfusion because of grade 3 WHO anemia, while support treatment with r-EPO, folic acid and vitamin E were sufficient for patient #2 to keep receiving Glivec regularly, without need of blood supply. The best known morbid effect of G-6PD deficiency is hemolysis induced by oxidative drugs. The patients present with different susceptibility to the hemolytic risk (a drug found to be safe in some G-6PD deficient subjects may not be equally safe in others) and when present the severity of hemolysis is almost always dose-related. Imatinib is a tyrosine kinase inhibitor and has been used with increasing frequency all over the world to treat chronic myeloid leukemia and other neoplastic disorders. Our two cases described presented evidence of imatinib-induced chronic non-spherocytic hemolytic anemia in glucose-6-phosphate dehydrogenase deficiency. Piperazine N-demethylate is found to be a metabolyte after administration of imatinib in humans and can be implicated in the oxidative event of the methemoglobin production that in G6PD patients can determine the onset of hemolysis. As our knowledge these are the first reports of hemolytic anemia in G6PD deficiency patients treated with imatinib. Although based on the description of only two cases that need to be validated in a larger series of patients, in our opinion imatinib must be given with caution in G6PD deficiency patients and we strongly suggest a preliminary test for G6PD activity in endemic areas.
PU339
GYNECOMASTIA AND HORMONAL ABNORMALITIES DURING IMATINIB (GLIVEC/GLEEVEC) ADMINISTRATION TO MALE CML PATIENTS
Gambacorti-Passerini C, Tornaghi L, Cavagnini F, Rossi P, Pecor-Giraldi F, Mariani L, Cambiaghi N, Corneo GM, Pogliani E, Gnessi L
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Imatinib mesylate (GLIVEC/GLEEVEC) belongs to the group of transduction signals inhibitors, inhibits the oncogenic tyrosine kinase BCR-ABL and has been recently approved as treatment for Chronic Myeloid Leukemia (CML). Additional targets of imatinib are c-Abl, platelet-derived growth-factor receptor (PDGFR) and c-Kit. C-Kit and PDGFR are receptor tyrosine kinases expressed in the testis and are involved in testosterone production. The safety profile of imatinib is still under investigation due to the short time of follow-up. We observed several cases of Gynecomastia in the CML male patients treated with imatinib prompting us to investigate hormonal modifications in this patients’ population. Objectives. Thirty-eight CML male patients enrolled in five different registrative or not registrative trials of Imatinib as monotherapy, were studied between November 1999 and May 2002 (mean follow-up 23.6 months, SD 7.5) with the objectives to evaluate them for gynecomastia, endocrine alterations, and changes in sexual life. Findings. Seven cases of gynecomastia developed (one grade 1 and six grade 2) (18%, 95% CI 6-30%) during treatment. A statistically significant difference between baseline and on-therapy values was found for free testosterone, total testosterone, progesterone and 17-OH-progesterone. Sexual dysfunctions emerged in six patients. A positive correlation was found between gynecomastia and hormonal abnormalities (combined low testosterone and high progesterone values; p=0.0045) and between hormonal abnormalities and treatment dose (400 mg vs >600-800 mg, p=0.0340). Conclusions. Our results show that in approximately 30% of males patients assuming imatinib for at least 6 months and studied here, develop gynecomastia and/or sexual dysfunctions which correlate with imatinib dosage and with changes in testosterone and progesterone plasma levels. Further data will be presented.

PU340
TRAUMATIC LEFT SHOULDER FRACTURE MASKING AGGRESSIVE GRANULOBLASTIC SARCOMA IN A CML PATIENT
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A 39 year-old man was diagnosed with Philadelphia Positive chronic myelogenous leukemia (CML) in chronic phase in 1989. He was treated with α-interferon at a median daily dose of 5 MU because of side effects (flu-like syndrome not controlled by the use of paracetamol) achieving a complete hematologic remission but no cytogenetic response. Neither related nor unrelated matched donor was found. In 1997, because of resistance to treatment, α-interferon was stopped and changed to hydroxyurea. In April 2000 the patient in chronic phase was enrolled in a perspective study on the use of the newly introduced tyrosine kinase inhibitor imatinib. He received 400 mg/day p.o. thereafter maintaining his hematologic response, but never achieving a cytogenetic response. In June 2002, left shoulder pain ensued; no scleletal or soft tissue lesions were evident at a bone X ray study. In August 2002, the patient was involved in a car accident which resulted in traumatic left humerus fracture which did not require surgical repair. Because of increasing left arm and shoulder edema suggestive of local thrombosis, sonographic and doppler studies were performed which were normal. In December 2002 MR imaging of the same shoulder showed, along with signs of the recent traumatic fracture, the presence of a proximal humerus osteolytic lesion associated with extensive substitutive tissue which was biopsied and lead to a diagnosis of granuloblastic sarcoma. Bone marrow aspirate was still consistent with a diagnosis of CML chronic phase. Imatinib dosage was increased up to 800 mg/day p.o. without response; therefore Cytosin-Arabinoside (500 mg/day i.v. for 4 days) and Dexamethasone (40 mg day e.v. for 4 days) were administered. Aplasia was complicated by the development of fever and TC imaging highly suggestive of mycotic pulmonary infection. Left shoulder lesion worsened even during post-chemotherapy aplasia and therefore local radiotherapy was administrated (21 Gray). The patient died in February 2003 with rapidly progressive local disease and mycotic pulmonary infection; however, bone marrow examination was still consistent with a diagnosis of CML chronic phase. Conclusions: Granuloblastic sarcoma, originating in a bone fracture, is a rare event; this localization was resistant to every kind of therapy and responsible for death, despite the persistent chronic phase of bone marrow.
PU341
SYSTEMATIC ASSOCIATION OF INTERFERON-α (IFN-α) AND HYDROXYUREA IN NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA PATIENTS: RESULTS OF A PILOT STUDY
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We conducted a pilot study by systematically combining IFN-α and HU at standard or maximally tolerated doses in newly diagnosed chronic phase CP-CML patients, to assess whether this treatment schedule could be more effective than IFN-α alone and less toxic than previously tested combinations. Twenty-two consecutive CP-CML patients (15 males and 7 females, median age 42 years, range 27-68) were included in the study. According to Sokal score, 17 pts were low risk (LR) and 5 intermediate (IR); according to Euro score, 16 pts were LR and 6 pts were IR. All patients were 100% Ph-chromosome positive. Patients initially received HU at a dose of 30-40 mg/kg/daily to reduce WBC < 15×10^9/L, and then IFN was added at increasing doses up to 5 MU/m^2; both IFN and HU were continued and adapted to keep WBC between 2 and 4×10^9/L. All patients achieved complete hematologic remission (CHR), in a median time of 6 weeks (range 2-16), with a mean HU administered dose of 0.48 g/day and a mean IFN dose of 5.4 MU/day. In 1 patient treatment was discontinued for serious neurologic toxicity. At a median follow up of 14 months (range 9-38), 21 patients were cytogenetically evaluable: a major karyotypic response (MKR) was observed in 13/21 patients (62%) with 9 (43%) being complete and 4 (19%) partial. No patients progressed to acute phase. Side effects > grade 2 were recorded in 54% of patients, with 27% discontinuing treatment. This pilot study, although conducted in a small number of predominantly LR-CML patients, indicates that a treatment schedule systematically combining IFN with HU can be as effective as other IFN combinations, but with less toxic effects. Combinatorial approaches incorporating these drugs with novel inhibitors with complementary mechanisms of action, deserves to be explored.

A 32 years-old woman was diagnosed with chronic myelogenous leukemia in August 1987. She underwent allogeneic bone marrow transplantation from an HLA matched related male donor on March 1988. Conditioning regimen included busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). Graft versus host prophylaxis included cyclosporine and short Methotrexate. No GVHD was seen. Post transplant follow up was uneventful and patient achieved full donor chimerism and cytogenetic and molecular complete remission soon after BMT. In September 2001 (14 years later) patient came to our observation in relapse in chronic phase. Cytogenetic studies demonstrated 97% metaphases XX Ph+ (host) and 3% XY Ph negative (donor). She was treated with Interferon and hydroxyurea until June 2002 without any modification of cytogenetics (residual normal donor metaphases 3%). In July 2002 (one year later, 15 years after transplantation) patient started treatment with Imatinib Mesylate at 400 mg daily. No significant side effects were noted and patient achieved hematologic remission in three months. After 6 months Imatinib therapy in situ hybridization demonstrated 99.7% Ph negative metaphases of male (donor) origin and 0.3% female Ph positive metaphases (host). In March 2003 her bone marrow was 99% male by in situ hybridization and 100% 46 XY by conventional cytogenetics. In June 2003 conventional cytogenetics was negative again and quantitative real time polymerase chain reaction detected a ratio of BCR-ABL/β2 microglobuline negative (0/50514). The patients remains in good health and has had no recurrence of GVHD. This case confirm efficiency of Imatinib Mesylate for chronic myelogenous leukemia patients relapsing after allogeneic bone marrow transplantation. Furthermore it enhances the capability of a minimal residual normal cells to completely repopulate bone marrow once pathological (Philadelphia positive) clone is suppressed (or possibly eliminate) by Imatinib therapy.
MOLECULAR PERSISTENCE OF BCR/ABL HYBRID TRANSCRIPT IN CML PATIENTS WITH COMPLETE CYTOGENETIC RESPONSE AFTER IMATINIB TREATMENT


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Imatinib-Mesylate (STI-571) has been demonstrated to induce complete cytogenetic response (CCR) in about 30-40% of CML patients. CCR is defined as disappearance of Ph chromosome-positive cells on all metaphases analysed in bone marrow (at least 20 mitoses are required). Despite these data, evidence of residual leukemic clones has been demonstrated by PCR analysis. We considered 21 CML patients who underwent regular evaluation of cytogenetic status and t(9;22) translocation molecular analysis after 6, 12 and 24 months of Imatinib treatment at conventional dosage. Therefore, we considered all the patients who obtained CCR and we analysed the correlation between CCR and molecular response. Molecular analysis was performed with reverse transcriptase polymerase chain reaction (RT-PCR) approach, to determine the hybrid transcript of bcr/abl fusion gene. First PCR assay, with a sensitivity of 10^{-3}, was performed on all samples, nested PCR assay, with a sensitivity of 10^{-4}, was executed only on samples negative in first PCR. In our casistic, we obtained 13 CCR: 3, 4 and 7 of them were respectively obtained 6, 12 and 24 months after Imatinib beginning. Contemporary to these responses, molecular analysis revealed persistence of bcr/abl hybrid transcript: 8 were positive in first PCR and 5 negative in first PCR became positive in nested. These data suggest that during Imatinib treatment, also in presence of CCR, leukemic clones continue to be present, maybe in a latent phase. These data suggest also that low burden of disease would be present, particularly in patients with only nested PCR positivity; for this reason quantitative PCR analysis should be required to obtain a better quantification of residual disease. Our findings suggest that cytogenetic analysis is useful in evaluating the response to Imatinib treatment. Considering the low sensitivity of this approach, other assays are required, particularly molecular quantitative assays may be recommended to determine minimal residual disease level.

CNS AND CUTANEOUS INVOLVEMENT IN PATIENTS WITH CML TREATED WITH IMATINIB IN HEMATOLOGIC COMPLETE REMISSION: 3 CASE REPORTS

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Imatinib mesylate (Glivec, Novartis) is a specific inhibitor of tyrosine kinase that selectively killed BCR-ABL cells. Clinical studies have shown that imatinib has a potent, oncogene-targeted action in all CML phases, although resistance to treatment appears to develop. However, little is known regarding both the distribution and the action of imatinib in extrahematologic sites (CNS, testis, skin, gastro intestinal tract) in humans, where resistant cells can develop and proliferate. In this report we describe three patients with CML treated with imatinib who developed CNS and in one case also cutaneous involvement while in complete hematologic remission (CHR) evidencing a weak antileukemic activity of the molecule in these sites. Case report #1. P.F., male, 72 years old was placed on imatinib while in accelerated phase achieving a rapid CHR. After 6 months of treatment he showed persistence of CHR and minor cytogenetic response but CNS lymphoid blasts were documented. Standard intrathecal treatment was started and the patient CNS blasts were rapidly cleared. Four month later, while still in hematologic remission, the patient noticed the presence of cutaneous lesions that a biopsy revealed to be a leukemic infiltrate. Two month later he progressed to bone marrow lymphoid blast crisis and died. Case report #2. M.M., male, 55 years old, was placed on imatinib also in accelerated phase. After 13 weeks of treatment he presented with CHR and major cytogenetic response but CNS lymphoid blasts were documented. Intrathecal therapy was started and the patient CNS blasts were rapidly cleared. Four month later, while still in hematologic remission, the patient noticed the presence of cutaneous lesions that a biopsy revealed to be a leukemic infiltrate. Two month later he progressed to marrow blast crisis three month later. Case report #3. L.M., female, 54 years old, started imatinib in chronic phase and CHR for interferon intolerance. After 7 months she was remaining in CHT and minor cytogenetic remission, but leukemic meningosis was evidenced. Shortly after she relapsed in the bone marrow too, was treated with systemic chemotherapy achieving complete remission, restarted imatinib, but four month later relapsed again in CNS while in CHR and minor karyotypic response. Intrathecal chemother-
apy and imatinib were restarted. The patient is still in CHR with cleared CNS and without blasts in bone marrow after 18 months follow up. These cases, despite the documented activity of the drug in humans and animals on CNS neoplasm, confirm that orally administered imatinib may have a low penetration into CNS suggesting careful monitoring and CNS prophylaxis in advanced disease. Interestingly, the first patient, who had no previous history of blastic phase, presented with uncommon CNS symptoms underlying massive infiltration of Ph-negative lymphoid blasts. The presence of Ph' negative lymphoid blasts in the patient N.1 could sustain the hypothesis of multi step clonal evolution of Ph'-independent CML cells facilitated by the low concentration of the Ph' inhibitor in this site. To our knowledge these are the first report of extrahematological localizations in CML patients treated with imatinib. Additional studies are required to identify imatinib 'sanctuary' sites or underlying specific resistance mechanisms to this revolutionary, but still not well known drug for CML patients.

PU345
THE PROGNOSTIC IMPACT OF ADDITIONAL KARYOTYPIC ABNORMALITIES TO THE PH' CHROMOSOME
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Karyotypic abnormalities additional to the Philadelphia translocation have been described at the time of blastic phase (BP) in the 75-80% of patients (pts) with chronic myeloid leukemia (CML). As a consequence, secondary evolution of the Philadelphia clone (clonal evolution, CE) is considered a negative prognostic sign by some Authors. In order to evaluate whether different chromosome changes are associated with specific clinical evolutions and even whether the time of their appearance could influence the prognosis, a cytogenetic study was performed on 101 CML patients at different stages of disease. Karyotype analysis was carried out on unstimulated 24-hours bone marrow cell cultures at diagnosis and every 3-8 months. Chromosome preparations were stained with Giemsa after trypsin treatment (GTG banding technique) and 25 metaphases were tentatively analysed in each examination: A clone was identified when the same hyperdiploidy and/or structural abnormality was found in at least two metaphases or the same hypodiploidy in at least three, according to ISCN raccomandation. At diagnosis 11 pts showed one or more karyotypic abnormalities additional to the Ph' chromosome. Variant or additional translocations were detected in 4 and 2 pts, respectively, -Y in 4, trisomy 8 in 1, i(17q) in 1, i(Ph') in 2 and 19p- in 1. Six patients achieved a major karyotypic remission (KR), 3 in response to IFN-α-based therapy and 3 to STI treatment. The other 4 did not obtained a KR and their survival was 10, 19, 32, 48 and 139 months, respectively. Two of these patients showed a further CE; one died 4 months after the appearance of +8, +Ph', 12p- and the other developed a duplication of Ph' 9 years after diagnosis, followed by a 6q- 2 years later. He died in BP 7 months later. A total of 27 mutational events were observed, after diagnosis, in other 22 pts. In two cases a second CE occurred 1 year after the first and was followed by a rapid BP and death. The third pt survived 10y, 8 after the first and 2 after the second CE. Coincidence between CE and clinical progression was seen in 6 pts. In the remaining 12 pts, +8 was observed in 7, while (Ph') in 1, t(3;9;22) in 1, -17 in 1, +Ph' in 1 and additional traslocation in 1. The median survival of these pts was 5y (range 1.3-14) with six pts still alive. Trisomy 8, duplication of the Ph' or Ph', and variant Ph'-translocations whenever occurring seems to be associated with a good prognosis. Instead monosomy of part or whole chromosomes, complex karyotype aberrations and multiple or subsequent CE appear to have a poor prognostic significance. Finally the lack of the Y seems to be associated with a good prognosis if present at diagnosis, but has a worse significance when occurring during the course of the disease.

PU346
PRESENCE OF TRISOMY 8 IN BOTH PH' NEGATIVE AND PH' POSITIVE CELLS IN A NEWLY DIAGNOSED CML PATIENT
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Cytogenetic clonal abnormalities in cell lacking detectable Philadelphia (Ph') chromosome and BCR-ABL rearrangements have been described in patients with chronic or accelerated-phase chronic myelogenous leukemia (CML) who were treated and responded to therapy with imatinib. Although less frequently, the phenomenon has been also observed in patients cytogenetically responsive to INF-α or INF-α-based treatments. This abstract reports the identification of trisomy 8 both in Ph'-positive and in Ph' negative cells in a newly diagnosed CML patient. The hematologic picture at diagnosis showed leukocytosis 155800/mm with a differential count (46% neutrophils, 19% lymphocytes, 23% metamyelocytes, 16% myelocytes and 5% myeloblasts).
consist of a myeloproliferative syndrome, anemia (7.2 g/dL Hb) and a platelet count (354x10^9 mm^-3) within the normal range. Systomatic enlarged spleen was also present. However bone-marrow pathology, did not show the typical features of CML. Cytogenetic examination carried out on 15 metaphases revealed the presence of trisomy 8 in both Ph'-negative (3 metaphases) and Ph' (12 metaphases) cells. Nesterd RT-PCR analysis for the BCR-ABL transcript showed the coexpression of p210 (b3a2) and p190 (e1a2). After a cytoreduction with HU, Imatinib treatment was started on October 2002. Despite increasing doses (400-800mg/day) of imatinib, a poor control of leukocytosis and splenomegaly was achieved. Thus, HU was added and subsequently substituted with a combination of INF-α and Idarubicine. A stable resolution of leukocytosis, but not of splenomegaly (2 cm below the costal margin) was observed on February 2003. Treatment was continued with imatinib (600 mg) in combination with 6-TG (80 mg/day) until March 2003 when it was stopped because of the occurrence of severe pancytopenia. At that time, cytogenetic analysis revealed the lack of the Ph-translocation and the molecular analysis the absence of the BCR/ABL transcript. However, the trysomy 8 was documented in all the 25 metaphases analyzed. After two months off therapy discontinuation, a resolution of leukopenia and piastrinopenia was detected. On May 2003, the patient was reevaluated at kariotypic and molecular level and the cytogenetic analysis confirmed the presence of only the clone with +8, but the BCR-ABL transcript was again detectable at the RT-PCR nested analysis. These data suggest that the expansion of a Ph'-negative clone may precede the acquisition of the Ph chromosome and that some cases of CML must be regarded as the result of a stem cell disease in which the occurrence of the Ph translocation represents a secondary rather a primary event.

PU347

CO-TREATMENT WITH BUTYRATE DERIVATIVES AND IMATINIB MESYLATE EXERTS A SYNERGISTIC EFFECT IN PH POSITIVE CELLS BOTH SENSITIVE AND RESISTANT TO IMATINIB

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Although Imatinib has been highly successful in the chronic phase of CML it is less so in the accelerated or blastic phases of the disease and in ALL, where remissions are less frequent, invariably of shorter duration, and followed by the emergence of a Imatinib-resistant disease. To identify novel molecules to inhibit Bcr-Abl with alternative strategy rather than to interfere at the ATP binding pocket level is a question urgently needed. Recently, SAHA has been demonstrated to enhance STI571 induced apoptosis in CML cells. We evaluated the effect of combination of Imatinib with two HDAC inhibitors, sodium butyrate and a stable prodrug xylitol derivative of butyrate (D1), on two different Ph positive cell lines, LAMA84 and KBM5, and on their respective STI571-resistant sublines, LAMA84R and KBM5-STI571 R1.0, representing two different models of acquisition of resistance to Imatinib. In fact, in LAMA84R the amount of the autophosphorylated fusion protein was increased in association with amplification of the BCR/ABL gene, whereas in KBM5-STI571 R1.0 the development of resistance was associated with the acquisition of a single C-T change at ABL nucleotide 944 (T315I) within ATP-binding pocket. The single agents and their combinations were studied for in vitro effect on proliferation by MT assay and the in vitro additive, synergistic, or antagonistic effects were then investigated by computer-assisted analysis using the CalcuSyn software. Both LAMA and KBM5 were sensitive to butyrates, with an IC50 of about 0.5 mM and 0.25 mM, respectively. On the contrary, LAMA84R and KBM5-STI571 R1.0 showed a cross resistance to both sodium butyrate and D1, with IC50 > 4 mM. Co-treatment with STI571 (0.125-1.0 µM) and sodium butyrate or D1 (0.125-1.0 mM) strongly inhibited cell proliferation, resulting in a marked synergistic effect in sensitive and especially in resistant sublines, as evaluated by isobologram analysis. In LAMA84 and LAMA84R inhibition of cell growth was mediated by induction of apoptosis, as demonstrated by Annexin V positivity and cell cycle analysis. Interestingly, in KBM5 Imatinib as single agent induced a blockade of cell cycle in G0/G1 phase, exerting a cytostatic rather than a cytotoxic effect, whereas co-exposure to Imatinib and butyrates resulted in a marked activation of apoptotic pathways, both in KBM5 and in the correspondent resistant subline KBM5-STI571 R1.0. We further evaluated the effect of a sequential treatment. Cells were exposed to low doses of butyrates (corresponding to IC20) for 24 hours, then washed and incubated with increasing concentrations of Imatinib for 72 hours. In LAMA84 and LAMA84R we observed a complete inhibition of cell growth at concentration of Imatinib as low as 0.25 microM. In KBM5 and KBM5-STI571 R1.0, pre-exposure to butyrate did not significantly enhance the effect of Imatinib. These findings show that Imatinib/butyrate combination may overcome clinical resistance to Imatinib, both when resistance is associated to an amplification of BCR-ABL gene or to a mutation within ATP-binding pocket.
MOLECULAR REMISSION OF PRETREATED CML PATIENTS IN RESPONSE TO IMATINIB


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The goal of therapy for CML is the complete eradication of Ph chromosome cells. Imatinib Mesylate (STI571, Glivec) has emerged as the most effective single agent for the induction of major cytogenetic response in patients with CML. Whether Imatinib alone or in combination can cure chronic phase CML is unknown, but only a little fraction of patients have become BCR-ABL-negative on the basis of PCR results. Since September 2000 we have treated with Imatinib Mesylate 23 patients with CML in chronic phase in our institution; they were intolerant or not responsive to interferon, pretreated with various lineage of therapy or untreated and therefore assigned to receive Imatinib alone or in combination with interferon. Three cases of chronic phase CML that obtained molecular remission with Imatinib are here described. The history of disease and the various types of treatment were quite different: the first patient (SP, M, 72 yrs) had received prior treatment with α-IFN for 18 months (interrupted for neurologic toxicity) and hydroxyurea for 20 months; the second (EF, F, 49 yrs) was treated at diagnosis with alpha-IFN alone for one year; the last patient (OD, F, 54 yrs) had received various treatments from 1995 to 2002 (alpha-IFN, hydroxyurea, autologous stem cell transplantation, IL-2 + GM-CSF s.c., activated LAK cells infusions). All three patients had obtained only a partial and transient cytogenetic response and were 100% Ph positive at the start of Imatinib, with p210 BCR-ABL transcript. They received Imatinib at the median dose of 400 mg, for a median time of 15 months (range 13-30). The higher hematologic toxicity encountered from Imatinib was grade 2 whereas the higher extra-hematologic toxicity was grade 1 (myalgia). All patients were monitored for cytogenetic response at intervals of three-six months, for a median follow-up time of 15 months (range 14-32). After a complete cytogenetic response was obtained, molecular assays were performed using reverse transcribed polymerase chain reaction (RT-PCR). Cytogenetic remission was achieved after a median time of 5 months (range 3-12) of Imatinib assumption and molecular response was demonstrated after a median time of 12 months (range 9-24). Pt n°2 showed a sustained molecular response (5 months), in the other cases further follow-up will be necessary to confirm the durability of the responses. In conclusion major and complete cytogenetic remissions in response to Imatinib are frequent and more over our limited and short-time experience demonstrates that the drug can be effective also in inducing molecular responses even in severely pretreated patients and with rare adverse effects. The duration of molecular response achieved with imatinib is still uncertain but it seems reasonable that improvements in survival will be seen. However given that very recent data demonstrated a failure of imatinib to kill cell cycle-arrested CML precursors, we must keep in mind that several escape/resistance mechanisms may arise even in chronic phase CML. Therefore, concurrent or sequential therapies (ex. α-IFN and Imatinib), based on different mechanisms (i.e. α-IFN immunotherapeutic effects), may be more effective in the treatment of CML.

COMBINATION IMATINIB AND PREDNISONE IN PATIENT WITH CHRONIC MYELOID LEUKEMIA IN BIPHENOTYPIC BLAST CRISIS

INDUCED HEMATOLOGIC AND COMPLETE CYTOGENETIC RESPONSES

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The causative event in the initiation of chronic myeloid leukemia (CML) is the formation of the BCR-ABL oncogene, molecular counterpart of Philadelphia chromosome (Ph), which codes for a constitutively active BCR-ABL Tyrosine Kinase. Imatinib Mesylate, formerly STI-571, inhibits the BCR-ABL Tyrosine Kinase with high selectivity and it has been demonstrated to induce clinical and cytogenetic responses in patients with CML. It is currently the frontline treatment for chronic phase, for patients in chronic phase resistant or refractory to interferon and for accelerated and blastic phase CML. The development of blast crisis in patient with CML is associated with very poor prognosis. Here we describe the occurrence of biphenotypic blast crisis following treatment with α-IFN therapy, in a patient with CML. In December 2000 a 65 year old male patient was diagnosed with chronic phase CML. Karyotypic and molecular analyses performed from bone marrow (BM) and peripheral blood (PB) cells showed t(9;22) BCR-ABL p210 (b3a2 type) and the simultaneous presence of t(8;21) AM L1-ETO. After cytoreductive therapy with hydroxyurea, α-IFN treatment began at a dose 9 MIU/day. Hematologic remission was obtained after the first month of treatment and major cytogenetic response was documented after one year. In May 2002, he showed hyper-leucocytosis (W.B. 40000/L) which was morphologically, immunophenotypically, cytogenetically and molecularly characterized as p210/p190 lymphoid and myeloid blast crisis. The patient was then treated with Imatinib alone for six months, at a dose...
variable from 200 to 400 mg/day with poor tolerance (severe hematologic toxicity), thus obtaining only a transient hematologic response. Because of a new hyper-leucocytosis the patient underwent polychemotherapy (Memorial: idarubicin 60 milligrams, aracytin 4.2 grams/daily for 5 days intravenously) with no clinical response. In March 2003 we started treating this patient with the combination Imatinib at a dose range of 600–800 milligrams daily plus PDN 1 mg/kg/day, achieving a complete cytogenetic conversion (Fish negative) after two months of treatment with only minor side effects; bone marrow molecular study revealed the presence of only p210 transcript. Thus, the combination Imatinib and PDN improved the clinical outcome of this otherwise unfavourable disease stage; moreover such a combination determined a higher quality of the cytogenetic response in comparison to previous treatments and allowed a better tolerance of Imatinib even at a higher dose. As it is unlikely to suppose that Imatinib can maintain the patient in this state, now we have addressed the patient toward an aploidentic allografting. Given that a reduced neoplastic cell load at the time of transplantation may improve survival, we believe that such a combination may be considered as a pre-transplant regimen for lymphoid blast phase CML and perhaps for relapsed Ph chromosome positive ALL.

PU350

NON TREATMENT-RELATED CHRONIC MYELOID LEUKEMIA AS A SECOND MALIGNANCY

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Treatment-related chronic myeloid leukemia (Tr-CML) is a rare event and its development seems to be associated to chemotherapy (CHT), radiotherapy (RT) and immunotherapy. However, to date Tr-CML cannot be distinguished biologically and clinically from de novo CML and, in contrast to secondary acute leukemia and myelodysplasia, peculiar cytogenetic abnormalities have never been reported in Tr-CML cases. Moreover, sporadic cases in which CML appeared following a neoplastic disease treated only with surgery, or without prior therapy, have also been described in literature, while nothing is yet known about non treatment-related CML (nTr-CML) as a second neoplasm. The characteristics of the very rare nTr-CML cases have never before been analyzed. The literature up to December 2002 was screened using the Medline database to identify cases of Tr-CML and nTr-CML: we found a total of 81 cases with secondary CML; among them, 4 (5%) were nTr-CML. Moreover, we considered 5 cases with nTr-CML identified among 270 newly diagnosed CML at our Department. Our report thus considers 9 cases with nTr-CML compared to 77 affected by Tr-CML as a secondary neoplasm. The median age at the appearance of the first tumor was compared in the two groups: it was 46 yrs in the Tr-CML group and 74 yrs in the nTr-CML group (p < 0.0001). This difference remained even when the patients in the Tr-CML group were subdivided according to previous therapy: CHT (p = 0.001), RT (p < 0.0001), and CHT plus RT (p < 0.0001). Hence, the median age at CML diagnosis was significantly higher in the nTr-CML than in the Tr-CML group (78 yrs versus 53 yrs, p < 0.0001, respectively). The median age of CML onset was confirmed to be higher in the nTr-CML patients even when they were subdivided according to the previous therapy regimen: CHT (p = 0.002), RT (p = 0.0004), and RT plus CHT (p=0.0002). Comparison of the latency period between the nTr-CML and the Tr-CML groups revealed no important difference, being 53 mo. versus 63 mo. (p = 0.3), respectively. No difference was observed even when the Tr-CML patients were divided into the three groups according to previous therapy (CHT, RT, and CHT plus RT). No significant difference was observed between the two groups in terms of male or female gender. The proportion of hematologic malignancies as first tumor type was not different in the two groups (44% in nTr-CML versus 56% in Tr-CML), although lymphoma was the most frequent first tumor among Tr-CML cases whereas no case in the nTr-CML group (p = 0.05). Our study underlines that nTr-CML as a second malignancy is a rare entity associated with elderly age. These data suggest that nTr-CML as a second neoplasm could be due to the effect of the immunological alterations produced by the first neoplasia, in addition to those distinctive to elderly age.
Anemias and Erythrocyte Disorders

PUJ51
UNRELIABLE ESTIMATION OF HBA1C DUE TO THE PRESENCE OF CAMPERDOWN HEMOGLOBIN ([ -104 (G6) ARG → SER])
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The measurement of glycated hemoglobin (HbA1c) in diabetic patients is a well-established procedure for disease management. HbA1c is a stable product of non-enzymatic glycosylation of hemoglobin β-chain by plasma glucose. The formation of glycohemoglobin is irreversible and its level in the red blood cells depends on the blood glucose concentration, exposure time to glucose and turnover of hemoglobin. Among glycohaemoglobin assays, High Performance Liquid Chromatography (HPLC) is the reference method. However, despite advances in the method's standardisation, several conditions such as blood loss, hemolytic anemias, chronic renal failure and hemoglobinopathies can lead to inaccurate glycated hemoglobin level determination. The aim of our report is to suggest the possible unreliability of HbA1c determination in presence of hemoglobin variants during routine metabolic evaluation.

Methods. We present a case of Camperdown hemoglobin, accidentally detected in a middle aged Italian man during metabolic evaluation for newly diagnosed diabetes. The hemoglobin variant has been identified by exchange high performance liquid chromatography (CE-HPLC) (VARIANT(c) HbA1c Program, Bio-Rad Laboratories, Hercules, CA, USA) usually performed for glycated hemoglobin. Molecular characterization of the mutation was achieved by DNA isolation from the WBC using standard methods followed by polymerase chain reaction (PCR) and by direct DNA sequencing on the PCR products. Results. A 56 year-old male of Northern Italian origin, presented to our Centre for a type 2 diabetes mellitus of recent diagnosis and HbA1c determination was routinely performed. At HPLC the patient's chromatogram showed an inappropriate peak of 38.5% in the HbA1c position suggestive for the presence of abnormal hemoglobin. Further evaluation identified an abnormal hemoglobin peak even higher (49.5%) eluting at 1.34 minutes in P2-window. Molecular characterisation of the mutation showed a nucleotide replacement, AGG → AGC at codon 104, causing the amino acid replacement Arg → Ser at position 104 (G6) that give rise to Hb Camperdown. Conclusions. The hemoglobin Camperdown described in this paper migrates on the chromatogram in the same position of glycated hemoglobin masking this latter's peak and making unreliable the estimation of the Hba1c fraction. Since more than 700 hemoglobin variants are known worldwide, it is necessary to consider the presence of abnormal hemoglobin in monitored diabetic patients with unexpected Hba1c values. Hemoglobinopathies can influence Hba1c determination thus their recognition is important in order to prevent inadequate diabetes management. If discrepancies in results are found on the HbA1c assay (either higher or lower than expected) hemoglobinopathy should be considered as a possible cause. In these cases we suggest the determination of fructosamine, since it is totally independent from hemoglobin although this is a marker of a shorter period of glycemic control (2-3 instead of 4-6 weeks).

PUJ52
RED BLOOD CELL DISTRIBUTION WIDTH VALUE IN DIFFERENTIAL DIAGNOSIS OF HYPOCHROMIC MICROCYTIC ANEMIAS
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Background. The red blood cell distribution width (RDW) (normal range = 11-16.5%), which provides a quantitative measure of the heterogeneity of red cell population in the peripheral blood, has been proposed as an additional variable used to distinguish heterogeneous thalassemia from iron deficiency. Objective. The RDW value in various hypochromic microcytic anemias was studied. Methods. The RBC indices (RBC count, MCV, MCH, RDW) were evaluated in 31 adults carrying β thalassemia traits (β-TT), with an age range of 18-60 years, in 37 subjects with iron deficiency anemia (IDA), aged 0-18 years, and in 53 healthy controls, with an age range of 25-45 years. Results. Although MCV and MCH values showed no statistically significant differences between β-TT and IDA, the mean RDW value was significantly higher in IDA than in β-TT (mean±standard deviation, 18±1.94% versus 14.88±1.77%, p < 0.001), while RBC was significantly high in β-TT than in IDA (6,100,000-7,200,000 versus 2,800,000-3,400,000, p < 0.001). Conclusions. RDW is the best index of iron deficiency, in fact it is a diagnostic parameter in 100% of patients with IDA. A significant rise of RDW, respect to
basal level (p < 0.01), in IDA patients after 5-7 days of parenteral therapy with iron was suggested as an important tool in differentiating IDA from β-TT. These observations could be kept in mind when establishing a differential diagnosis between β-TT and IDA by determining only the red blood cell count and red cell indices.

PU353
SEVERE HEMOLYTIC CRISIS WITH MULTIPLE ORGAN FAILURE AFTER PARACETAMOL TREATMENT IN A PATIENT WITH G6PD DEFICIENCY
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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common of all clinically significant enzyme defects in the World. The enzyme deficiency is inherited with X-linkage. More recently, it has been found that G6PD is one of a cluster of genes on the distal long arm of the X chromosome and are known more than 400 variants and 100 mutations. The most common manifestations of G6PD deficiency are rare episodes of hemolysis due to oxidant drugs administration during infections, fava bean ingestion or infants neonatal jaundice. Chronic nonspherocytic hemolytic anemia is present in rare cases. Clinical case: A twentysix year old man with a negative anamnesis, after one week of high fever, was treated at home with quite high doses of Paracetamol (4 g/d - total dose 25 g) and in the three days before admission to Hospital he was treated also with Amoxicillin (2 g/d- total dose 6 g) and Clavulanic acid. He was admitted to a surgical unit for severe jaundice and after few hour to intensive care unit for worsening of the clinical condition with severe anemia, hepatomegaly, splenomegaly, acute renal and hepatic failure. Laboratory findings: Hb 7.1 g/dL, WBC 7100/cmm, Plt 72000/cmm, Creatinine 6.14 mg/dL, LDH 5770 U/L, bilirubin 57 mg/dL, ALT 126 U/L, AST 552 U/L. A fine needle liver biopsy evidenced normal lobular structure and high hemaphagocytic activity. Bone marrow biopsy showed erythroid hyperplasia with histiocytes and phagocytosis of red cells. After transfusional and immunosuppressive (Prednisone) treatment, in association with renal ultrafiltration, a slow and progressive improvement of the hepatic and renal function was seen accompanied by hematologic recovery. Further investigation evidenced G6PD deficit (0.14 U/gHb - normal range 0.01±0.1)unknown to the patient. The molecular test identified variant G6PD Santamaria mutation (542 A→T/ 376 A→G)in the patient and in his mother. The steroid therapy was slowly tapered and after three months a complete recovery of hepatic, renal function and blood count was observed, remaining only mild splenomegaly. Discussion: The mechanism by which drugs produce hemolytic anemia seems to be due to oxidative injury on the erythrocyes, with necessity of the activation of the phenososphosphatic pathway blocked by the G6PD deficit, this causes hemoglobin denaturation and reticuloendothelial system capture. Although Paracetamol is considered a safe drug at therapeutic dosage in patients with G6PD deficit, rare case of hemolysis were described in suicidal subjects and/or with alcohol abuse. The patient was treated with dosage of Paracetamol higher then normal and for long time, this treatment associated with an infectious process, may worse oxidative stimulus. Hepatic biopy did not evidence the lobular-center necrosis (direct damage) but a hemophagocytic pattern, therefore this seems to seggest that the hepatic damage was due to the defect in G6PD activity. In conclusion paracetamol must be used with caution and for short time in case of G6PD deficit.

PU354
SERUM LEPTIN LEVELS IN PATIENTS WITH SIDEROPENIC ANEMIA AND IDIOPATHIC THROMBOCYTOPENIA
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Leptin is a 16 kDa protein secreted from white adipocytes. In rodents and in human, it seems to be implicated in the regulation of food intake, energy expenditure and basal metabolism. Recently, leptin has been demonstrated also to stimulate hematopoietic stem cells in vitro. The aim of this study was to assay the leptin level in hematologic patients to demonstrate an eventual existence of a serum alteration. We studied 21 patients affected with sideropenic anemia (SA), 18 with idiopathic thrombocytopenia (IT) and 39 healthy subjects as the control group (CG). Hemocromocitometric examination and determination of serum leptin levels were performed two times: the first one, at the time of diagnosis for patients and of enrolling in the study for CG; the second one, after the normalization of Hemoglobin (> 12 g/dL) for SA and after one year of therapy in IT. The relationships of serum leptin levels to serum erythropoietin (Epo), serum thrombopoietin (Tpo) levels and blood cell parameters were also studied to analyze their variations in these subjects. Our results shown no statistically significant differences in the serum leptin levels between the three groups in both determinations. Moreover, the leptin levels in SA were not modified by the treatment of ane-
Cryoglobulinemia is still unknown. Recently, the availability of rituximab, a chimeric monoclonal antibody directed against the B-lymphocyte antigen CD20, for its efficacy in the lymphoid tissue together with its good safety profile, have encouraged investigations of the therapeutic activity of this drug in some autoimmune disorders with a prevalent autoantibody-based pathogenesis. In particular autoimmune cytopenias, mixed cryoglobulinemia and some other autoimmune disease have been investigated with interesting preliminary results. In type II mixed cryoglobulinemia, usually associated with HCV infection, there is a vasculitis due to the cryoglobulin production sustained by the clonal non-neoplastic proliferation of rheumatoid factor-positive B cell clones. The hypothesis that rituximab could act selectively on cryoglobulinemic B-cell lymphoproliferation is at the base of its employment in this kind of disease. We describe the case of a 53 years old man affeted from type II mixed cryoglobulinemia associated with hepatitis C. The initial treatments, first with prednisone and then with cyclosporin-A, didn’t reach a satisfactory response, while a partial control of disease was obtained with azatoprine. In October 2002 the patient developed an important pericardial effusion, severe anemia and moderate splenomegaly. The laboratory tests showed a little monoclonal component IgM/K and the bone marrow aspirates documented a B-lymphocyte monoclonal infiltration of about 3%. In December 2002 was started a treatment with rituximab 375 mg/m² i.v. once a month plus Chlorambucile 4 mg/day×14 days every month. After 6 course we have noted an important improvement of the clinical picture with the normalization of the renal function, of the Hb level associated to the reduction of the size of the spleen, of the serum monoclonal component and of the cryoglobulins level. No particular toxicity was noted. This case shows the utility of the employment of rituximab plus low-dose of chlorambucile in HCV related type II mixed cryoglobulinemia associated with the presence of clonal B-lymphocyte proliferation.

PU355
EMPLOYMENT OF ANTI-CD20 (RITUXIMAB) PLUS CHLORAMBUCILE IN THE THERAPY OF THE HCV RELATED CRYOGLOBULINEMIA
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Red blood cells are normally smaller than reticulocytes. The only known exceptions are found in: a) pernicious anemia being treated with vitamin B12; b) the development of iron deficiency; c) erythropoietic regeneration in bone marrow transplants. Contrary to manual scanning, the reticulocytes must be sphered to permit automatic counts. However, the most recent Coulter hematology analysers (GEN-S, LH 750), coupled with other reticulocyte parameters, lead to a new parameter, MSCV. As reported by other authors (2), MSCV presents a lower MCV value in hereditary spherocytosis than in normal subjects. This occurs because a hypoosmotic reagent is added to the reticulocyte count, thus sphering the erythrocytes. Although normal red blood cells can expand in low osmolarity conditions, in hereditary spherocytosis spherocytes reach a critical osmotic volume. Moreover, since they have membrane defects they fragment consistently, leading to a drop in MCV values. Instead, in acquired spherocytes with therefore no membrane defects, reticulocyte volume thus determined is normal. Other commercially available hematology analysers count reticulocytes by sphering them, but they do not determine MSCV. This new parameter, which is present only in hereditary spherocytosis spherocytes, thus leads to a new test that, coupled with other specific assays, aids in the identification and confirmation of hereditary spherocytosis. The diagnosis of spherocytosis can present significant difficulties, because from a clinical standpoint there are mild or asymptomatic forms. Above all, however, there is no pathognomonic laboratory test. MCHC, osmotic resistance and the erythrocyte membrane tests can all lead to false negative results for a number of

PU356
SPHEROCYTOSIS AND ELLIPTOCYTOSIS: A NEW DIAGNOSTIC PARAMETER
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The best therapy for the HCV related cryoglobulinemia is still unknown. Recently, the availability of rituximab, a chimeric monoclonal antibody directed against the B-lymphocyte antigen CD20, for its efficacy in the reduction of B-lymphocytes in the peripheral blood an the lymphoid tissue together with its good safety profile, have encouraged investigations of the therapeutic activity of this drug in some autoimmune disorders with a prevalent autoantibody-based pathogenesis. In particular autoimmune cytopenias, mixed cryoglobulinemia and some other autoimmune disease have been investigated with interesting preliminary results. In type II mixed cryoglobulinemia, usually associated with HCV infection, there is a vasculitis due to the cryoglobulin production sustained by the clonal non-neoplastic proliferation of rheumatoid factor-positive B cell clones. The hypothesis that rituximab could act selectively on cryoglobulinemic B-cell lymphoproliferation is at the base of its employment in this kind of disease. We describe the case of a 53 years old man affeted from type II mixed cryoglobulinemia associated with hepatitis C. The initial treatments, first with prednisone and then with cyclosporin-A, didn’t reach a satisfactory response, while a partial control of disease was obtained with azatoprine. In October 2002 the patient developed an important pericardial effusion, severe anemia and moderate splenomegaly. The laboratory tests showed a little monoclonal component IgM/K and
reasons. Based on our experience, which we have gained by studying various members from different generations of a single family with hereditary spherocytosis, MSCV was always lower than MCV and, in equivocal cases, we conducted additional tests (glycerol test instead of osmotic resistance, etc.). Like spherocytosis, also elliptocytosis is a hereditary hemolytic anemia caused by a membrane defect. Consequently, we decided to investigate if MSCV showed a similar behaviour here as well. Our data reject this hypothesis: in elliptocytosis, erythrocyte spherering evidently does not lead to cell membrane loss. All of this reinforces the diagnostic value of MSCV in spherocytosis. Our case studies are reported in Table 1. Despite diagnostic uncertainty, in both cases there is no doubt about the therapeutic approach: splenectomy should be performed on all anaemic patients and patients with a history of hemolytic or aplastic crises. Since this approach is irreversible, it should be performed based on the most confident diagnosis possible. The addition of this new parameter can aid us in this sense. Since hemograms and reticulocyte assays have now become routine tests, an MSCV test result lower than an MCV should permit early diagnosis and, in the future, help avoid diagnoses in adult subjects, as is sometimes still the case.

References


Background. Hemolytic disease of the newborn (HDN) is triggered by maternal IgG, which, passing through the placental barrier, binds the fetal erythrocytes that thus suffer accelerated destruction. The clinical severity of HDN can vary from intrauterine death to only serologic abnormalities detected in an asymptomatic newborn. Alloantibodies, bound to the erythrocyte membrane in vivo, can be detected in vitro by the direct antiglobulin test (DAT), which is the main laboratory test to diagnose HDN. Design and methods. According to international guidelines to prevent HDN, in our laboratory, ABO and Rh typing of newborns and respective mothers (to make evident a possible incompatibility), DAT on cord blood and the indirect antiglobulin test (IAT) on maternal blood (to detect antibodies in serum) are daily performed by microcolumn techniques (Gel test). In the period from 1999 to 2002, over 16,000 newborns and respective maternal blood samples were analyzed according to the previous protocol. Case report. We have found 5 cases of ABO foetal-maternal incompatibility (all the mothers of group 0; 3 newborns of group A and 2 B) with negative DAT and IAT. In our experience, similar cases occur frequently, indicating an antigenic incompatibility without hemolysis, therefore no diagnosis was made. Nevertheless these 5 newborns shown the clinical findings of HDN (anemia and hyperbilirubinemia), so a deepening of laboratory investigation needed. Maternal sera were treated with 2-mercaptoethanol (2-ME) to remove natural agglutinin (IgM) and to confirm the presence of IgG anti-A or anti-B. Their titles ranged from 1:1.024 to 1:4.096. The antibody elution from washed and concentrated cord red blood cells was performed and the suspected anti-A/B specificity was demonstrated, confirming the diagnosis of HDN. The IgG subclass of detected antibodies was IgG3 in all the cases. At the end, suffering newborns were cured by phototherapy for 2 days. Conclusion. Our study demonstrates that, in case of patient with negative DAT and clinical features strongly suggesting hemolysis, the eluate from concentrated red cells is more sensitive than direct study of RBC. In fact, the four IgG isotypes show structural and functional differences; therefore the amount of RBC-bound IgG3, the subclass with the greatest hemolytic ability, can be sufficient to cause hemolysis in vivo, but inadequate to determine a positive DAT in vitro. In conclusion a neg-
ative DAT cannot exclude an immuno-mediated RBC injury and the management of a hematologic patient must be evaluated combining clinical condition and laboratory data.

PU358
SOLUBLE TRANSFERRIN RECEPTOR AND FERRITIN IN PATIENTS WITH ANEMIA
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Serum soluble transferrin receptor (sTfR) has been introduced as a new tool for the diagnosis of iron depletion. It is mainly useful to distinguish iron deficiency anemia (IDA) from anemia due to chronic disorders (ACD). In this study, we compare the differential diagnostic values of sTfR and Ferritin in anemic diseases. One-hundred and eighty subjects, divided in four groups, have been studied. Eighty-three healthy subjects compound the control group (CG); 54 patients were affected by IDA, with Ferritin < 20 ng/mL in females and < 40 ng/mL in males; 34 patients affected by ACD, diagnosed according to Bentley’s criteria; 9 patients with β-thalassemia trait (TT), diagnosed by hemoglobin electrophoresis. sTfR, Ferritin and blood count were assayed in all subjects. The highest value of sTfR (7.39±1.87 mg/L, mean±SD) was found in IDA patients, while the lowest in ACD (3.0±0.8 mg/L). Ferritin was significantly lower in patients with IDA than in other patients or in CG (p < 0.05). In IDA, significant correlations were found between Ferritin and hemoglobin (p=0.002) and between sTfR and hemoglobin (p=0.015). IDA patients were treated with oral iron for 12 weeks; after therapy, hemoglobin sTfR and Ferritin were assayed again. The decrease of sTfR did not occur so rapidly as the increase of serum Ferritin after iron repletion. In fact, the change of sTfR was more significant (p < 0.001) than of Ferritin (p < 0.05). In this study, sTfR was significantly highest in IDA than ACD and CG, while it was not significantly different between IDA and TT. Thus sTfR could be used to distinguish IDA from ACD as reported by others authors, while Ferritin is more useful than sTfR in indicating iron repletion after therapy.
Background and Objectives. Bone disease is an important cause of morbidity in patients affected by major Thalassemia, is a multifactor disease: massive bone marrow expansion, delayed puberty, hypogonadotropic hypogonadism, other hormonal disfunctions (hypothyroidism, hypoparathyroidism, diabetes mellitus), iron overload and deleterious effects of chelant therapy on osteoblasts. Aims of the study are evaluate the prevalence of reduction of mineral bone mass in thalassemic patients and analyze the predisposing causes. Materials and Methods. Mineral Bone Density (BMD) of lumbar spine (L2-L4 level), femoral neck and distal radius have been evaluated by dual-energy X ray absorptiometry (DEXA) (UNIGAMMA RAY-PLUS) in 31 patients affected by major thalassemia (12 males and 19 females) age averaged 24.5 (range 15-38). Anamnestic and clinical data of all patients have been collected underlying the risk factors of reduction of bone mass, the patients affected by diabetes type I and drug induced puberty, although not statistically significant, are numerically relevant.
PU363
EVALUATION OF HEMOGLOBIN F CONTAINING RED BLOOD CELLS (F+ CELLS) BY A FLOW CYTOMETRIC TECHNIQUE IN PEDIATRIC PATIENTS WITH HOMOZYGOUS SICKLE CELL DISEASE IN THE DEMOCRATIC REPUBLIC OF CONGO

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Sickle cell disease (SCD) is an inherited hemoglobin disorder characterized by chronic hemolytic anemia, recurrent painful episodes and a large clinical heterogeneity, due to different haplotypes generated by different hemoglobin gene mutations, as well as to psychosocial and environmental influences. Clinical manifestations in SCD are also influenced by percentage of Fetal hemoglobin (HbF) in patients' blood (high levels of HbF are associated with less severe clinical manifestations and a lower number of painful episodes). In this study we report the percentage of red blood cells containing HbF (F+ cells) in SCD patients of Bantu haplotype and controls measured by flow cytometry. A total of 89 pediatric peripheral blood samples collected in Kinshasa (CDR) were analyzed in this study: 42 of these samples were from homozygous SCD patients while 47 were from non-homozygous SCD patients and therefore used as controls. The analysis was performed on heparin preserved samples stored at 4°C for no more than two weeks before studies. In addition, we included 5 peripheral blood (PB) samples and 4 cord blood (CB) cells samples from normal healthy donors used as negative and positive control, respectively. All samples were fixed and permeabilized by Glutaraldehyde and Triton X-100 and incubated with a MoAb-HbF-FITC. Thereafter, a flow cytometric technique was utilized for calculating the number of F+ cells. The results were expressed as percentage of F+ cells respect to total number of RBC of each sample. The positive cut off point for F+ cells was set at 0.5% above negative population of isotype control staining cells. Among the 5 PB samples of normal healthy donors, the% of F+ cells was above 0.5% only in one sample in whom F+ cells were 2.08%. Samples from CB had a% of F+ cells ranging from 12.13% to 46.58%. The mean%±SD of F+ cells in 42 SCD samples was 5.44%±7.6 (median: 2.19%; range 0-30.3%); in particular 40/42 (95%) SCD patients showed a% of F+ cells >0.5%. HbF expression was also analyzed using the Kolmogorov-Smirnov statistic test (D-value), which allows objective and accurate identification of small differences in fluorescence intensity. Sample with a D-value >0.15 were considered positive. The mean D-value±SD of 42 SCD samples was 0.21±0.007 (median: 0.33; range 0.07-0.57) and 40/42 (95%) SCD patients had a D-value >0.15. In the 47 samples of the control population, the mean%±SD of F+ cells was 0.50%±1.06 (median: 0; range 0-5.18%), with 12/47 (25.5%) values above 0.5%. The mean D-value±SD of 47 control samples was 0.024±0.034 (median: 0 range 0-0.15), no one of the patients had D-values >0.15. Correlation among F+ cells% and D-value for the entire population was highly significant with a r = 0.67 (p<0.0001). The comparison among SCD patients and controls as for the% of F+ Cell and D-value by mean of the Mann-Whitney U-test was also highly significant (p<0.001 for both F+ cells% and D-value). In conclusion, flow cytometry is a useful and more precise techniques for detecting F+ cells
in SCD patients. Moreover, the D-value is able to discriminate better between normal and pathologic population. Furthermore, this technique may allow a correct monitoring of the therapeutic effect of Hydroxyurea on HBF levels in SCD patients. This is important considering that high levels of HBF are associated with less severe clinical manifestations and a lower number of painful episodes.

PU364
ERYTHROEXCHANGE WITH CELL SEPARATOR IN DREPANOCITICS PATIENTS USING DOUBLE SDR (SINGLE DONOR RED CELLS-MC):
A WAY TO REDUCE HEMOGLOBIN “S” AND FERRITIN LEVEL
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Vaso-occlusive disease and recurrent infections are very frequent in drepanocytic patients. Chronic transfusion therapy reduces Hemoglobin S level and generally prevent recurrent vaso-occlusive disease but simultaneously determines a progressive increase of ferritin level that need a regular chelation therapy with poor compliance from patients. Erythroexchange is an alternative method to maintain a low Hemoglobin S level and prevent ferritin level increase but the use of standard red blood cell for transfusion therapy don’t resolve the infectious risk and the incidence of allo-immunization due to high transfusional exposure. Trying to reduce transfusional risk but maintaining the advantage of procedure we made the erythroexchange using double single donor red cells (SDR). The erythroexchange was done with manual method or with automatic cell separators that reduces procedure time. Now we show the data of 10 patients that regularly performed erythroexchange with automatic cell separators using double single donor red blood cell (SDR), comparing them to patients subjected to chronic simple transfusional therapy and manual or automatic erythroexchange using standard red blood cell regarding number of transfusions, alloimmunization occurred, number of vaso-occlusive episodes and quality life. This preliminary analysis would establish the advantages, the complications and efficacy of erythroexchange with automatic cell separators using double single donor red blood cells (SDR) in patients that otherwise were chronically transfused or subjected to manual erythroexchange using standard red blood cells to avoid vaso-occlusive disease or recurrent infections. Results: Erythroexchange with automatic cell separator using double single donor red blood cells or standard red blood cells was well tolerated and the levels of ferritin were stable in patients subjected to automatic or manual erythroexchange, without concomitant chelation therapy, whereas ferritin level increased in chronically transfused patients. None of patients developed new vaso-occlusive episodes, maintaining level of hemoglobin S before erythroexchange and before transfusion below than 60%. Nevertheless erythroexchange with cell separator using standard red blood cells required greater transfusional exposition than cronical transfusion or manual erythroexchange exposing the patients to higher transfusional risk. Erythroexchange with automatic cell separator using double single donor red blood cells, instead, showed a lower transfusional risk but maintain the advantages of erythroexchange with cell separator. Moreover the analysis of recovery days showed a 50% reduction in patients subjected to erythroexchange with automatic cell separators, improving their quality life. Conclusions: We think, for this preliminary experience, that erythroexchange is safe and effective method to control hemoglobin S levels, to limit iron accumulation, to reduce vaso-occlusive disease and to meliorate the quality life of drepanocitic patients that present severe vaso-occlusive episodes. We think also that using standard red blood cell the trasfusional risks are very high. The erythroexchange with cell separator using double single donor red blood cells (SDR) is a safe method to reduce transfusional risk and maintain advantages of procedure.

PU365
SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN INSULIN DEPENDENT DIABETES MELLITUS (IDDM) ANEMIC PATIENTS
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In a previous report, we observed impaired Erythropoietin (EPO) production in Insulin Dependent Diabetes Mellitus (IDDM) anemic patients. This finding is present also in very early stages of renal involvement, when proteinuria is the only sign of diabetic nephropathy. It is not clear why diabetic patients differ from other nephropatic patients who present a blunted Epo response to anemia much later. Our results are in accordance with other Authors’ observations. Vascular endothelial growth factor (VEGF) is one of the most important cytokines involved in embryonic vasculogenesis and angiogenesis. Many reports focused the relationship between VEGF and the vascular complications of IDDM. Hypoxia is a potent stimulus for VEGF as far as for EPO production. Many experimental evidences demonstrated that the pathway of the hypoxia induced production is the same for VEGF and EPO. In two series of neoplastic patients, VEGF was higher in anemic...
patients in comparison to those with normal Hgb levels, perhaps, as a consequence to tissue hypoxia. We examined 47 IDDM diabetic patients (21 M; 26 F; aged 23-71, median 37); 35 of them had Hgb 8805;11.5 g/dL (non anemic diabetes-nAD) (13.1±2 g/dL) and 12 had Hgb value under this level (Anemic Diabetes-AD) (10.7±0.7). CBC, serum EPO and VEGF (ELISA-R&D System), the most important serum parameters were assayed. Forty-two blood donors (18 M; 23 F) entered the control group. The purpose of the study was to verify if our diabetic patients have an overproduction of VEGF and if so, how anemia influences the VEGF values. The results obtained can be summarised as follows: i) as previously reported, AD patients have a blunted EPO response to anemia (9 out of 12 patients; 75%); ii) 7 out of 12 anemic patients presented diabetic neuropathy and retinopathy; iii) the VEGF in all diabetes patients is higher than in control group: 350.9±249.9 pg/mL versus 176±157.8 pg/mL; p<0.0001; iv) the VEGF in AD group is lower than in nAD group (237.9±58.4 pg/mL vs 389.7±233 pg/mL; p=0.04) and comparable to control group values (176±157.8; p=n.s.); v) VEGF values are not correlated to any diabetes complications. Many hypotheses were formulated to explain the early EPO production impairment observed in diabetic patients such as urinary loss of the hormone caused by proteinuria, early fibrosis of kidney interstitium, low adrenergic stimulation, perhaps, as a consequence to tissue hypoxia. We examined 62 women affected by Eating Disorders (EDs): 30 anorexia nervosa (AN), 27 bulimia nervosa (BN), 5 eating disorders no other specific (EDNOS). Thirty healthy women entered the control group. Body Mass Index (BMI), complete blood count (CBC) and differential, Reticulocytes, serum iron, TIBC, ferritin, vitamina B12 and folate, the most important laboratory parameters and Hamilton depression rating scale (21 items) score (HDRS21) were assessed. The aim of this study was to give a new insight on hematologic features in EDs; secondly, to clarify if a difference of hematologic and/or hematocochemical parameters is present between AN and BN. Hgb, Hct, MCHC, TSI and WBC are significantly lower in patients (all together) as far as the absolute neutrophil, monocyte and lymphocyte counts while Red Cell Distribution Width (RDW) and HDRS21 score are higher. Considering AN and BN in separate groups, some differences were observed: in BN, only neutrophils are lower than normals, the anemia is more frequent (even though without statistical significance) and the Red Blood Cell (RBC) indices and Transferrin Saturation Index (TSI) indicate more clearly an iron deficient status in comparison to anorexic. These results are summarized in the table 1. Only in BN patients RDW shows an inverse correlation to ferritin (p=0.047) and the frequency of low ferritin values is higher in BN than in AN (p=0.027). Moreover, in all patients, BMI correlates directly with WBC (p=0.037), Lymphocytes (p=0.009), RBC (p=0.001) and Reticulocytes (p=0.047) and inversely with MCV (p=0.005), MCH (p=0.005) and ferritin (p=0.031). No significant difference is present for vitamin B12 and folate. HDRS21 score is higher in BN than in AN but shows a significant correlation only with the length of the disease. We conclude, that WBC count and lymphocytes, in particular, are strictly related to the nutritional status. Anemia is not very frequent and severe in EDs. Nevertheless, two different erythropoietic patterns can be recognised. In AN erythropoiesis is very little compromised perhaps for the reduction of body demands (low BMI, loss of menstruation). On the contrary in BN anemia, erythrocyte indices alterations and a poorer iron status are present indicating the unbalance between the nutrition defect and normal body demands (normal BMI and menstruation).
Reticulocyte mean corpuscular hemoglobin (CHr) gives information on hemoglobin synthesis and on iron availability (as a parameter more stable than ferritin or transferrin saturation), and is a very early indicator of iron therapy efficacy. Some limitations exist, attributable to its unique normal value (27 pg) proposed by a few researchers, which cannot be satisfactorily applied in case of micro or macrocytosis. We tried to improve the capacity of this index to classify anemia by the application of dynamic reference values (CHr-a). We studied 266 subjects: 207 normal controls, 58 anemic patients (42 hypochromic, 5 normochromic, and 11 hyperchromic anemias) and a single patient with iron deficiency anemia during iron supplementation. CHr was calculated as follows: CHr = CHCMr × MCVr, where mean corpuscular hemoglobin concentration of retics and mean corpuscular volume of retics were directly measured by the H3-Bayer analyzer. Then, we considered the difference between the calculated CHr and the CHr expected for the MCVr of the subject studied (CHr-α) (δ-CHr= CHr-CHr-α). CHr-a was calculated as follows: as CHr and MCVr are in direct proportion and as CHr= CHCMr × MCVr identifies a straight line passing through the origin of orthogonal axes, we constructed a line (Figure) taking into consideration mean CHr and MCVr values of the 207 normal subjects. Then we calculated each CHr-a for each MCVr included in the interval between 45÷150fL. If we report on the orthogonal axes each CHr and MCVr of the 58 anemic patients, three clusters are identifiable: on the left of the line (hypo), on the right of the line (hyperchromic) and on the line itself (normochromic). This distribution is even clearer if we consider δ-CHr values, which is higher than, equal to or minor than zero in hypochromic, normochromic or hyperchromic anemia, respectively. Furthermore, CHr gives information more accurate than ferritin during iron therapy. The single patient we studied continued to take iron, taking into account the persistence of negative δ-CHr, although he had attained normal ferritin and hemoglobin levels, and hemoglobin further increased and then stabilized when δ-CHr was equal to zero. In conclusion, in anemic syndromes δ-CHr easily allowed the identification of three groups (hypo, hyper and normochromics) and was more helpful than ferritin to address iron supplementation.

A 80 yr male, affected by chronic renal failure, was started on recombinant human erythropoietin (rHuEPO) at a weekly dosage of 4.000 U, subcutaneously, because of progressive anemia, on January 2002. He initially showed a favourable response to rHuEPO, but, on August 2002, still on rHuEPO, his Hb level unexpectedly dropped, and he required packed red cell transfusions. Common causes of rHuEPO failure (i.e.: iron deficiency, infection, flogosis, bleeding) were excluded. rHuEPO dosage was increased up to 4.000 U/3/week, without any response, and persistence of transfusion need. On September 2002 rHuEPO was definitively stopped, and a regular maintenance transfusion regimen was planned. At that time, normochromic normocytic anemia was present, and reticulocytes were severely reduced (0.2%). Other causes of anemia (i.e. hemolysis, iron deficiency, folate or B12 deficiency, bleeding) were excluded. The bone marrow aspirate and biopsy showed a picture of Pure Red Cell Aplasia (PRCA). B19 Parvovirus infection was also ruled out. On the basis of these findings, the rare and recently described (Casadevall N et al., N Engl J Med, 2002) PRCA caused by anti-erythropoietin antibodies was suspected. The search for anti-erythropoietin antibodies (immunoprecipitation test) was positive, and confirmed our diagnostic assumption. Because of advanced renal failure, the more common immunosuppressive treatments, (i.e. corticosteroids, cyclosporin or cytotoxic drugs) were not feasible. On the basis of recent favourable result obtained in several autoimmune disorders (Zaja F et al., Haematologica, 2002), the patient was started on Rituximab (375 mg/m2/week, for 4 times).
on January 2003. After the end of the last dose of Rituximab, rHuEPO was started again, at a weekly dosage of 8,000 U, intravenously. Reticulocytes gradually increased up to 7% after 2 weeks of rHuEPO treatment, and the last transfusion was performed on March 2003. From then on, the Hb level steadily increased up to 11.9 g/dL on April 2003, and the patient, at the time of writing, is still free from transfusions, still receiving the same dose of rHuEPO i.v. On May 2003, the bone marrow aspirate showed a marked increase of erythroblasts, and CD20+ cells, either in bone marrow or in peripheral blood, were still absent.

PU369
CYCLOSPORIN-A (CS-A) FOR REFRACTORY COOMBS-NEGATIVE AUTO-IMMUNE HEMOLYTIC ANEMIA
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A 35 year old female came at our observation on November 2000. She had been successfully treated in 1991 with steroids for auto-immune neutropenia. In the subsequent period the hematologic parameters had been normal. On November 2000, she was referred to another institution because of fatigue, jaundice, and low grade fever (37.5°C). The laboratory data showed: severe anemia (Hb 3.5 g/dL), (MCV 92.9 mm3) with a low grade fever (37.5°C). The laboratory data showed: severe anemia (Hb 3.5 g/dL), (MCV 92.9 mm3) with a low grade fever (37.5°C). The laboratory data showed: severe anemia (Hb 3.5 g/dL), (MCV 92.9 mm3) with a low grade fever (37.5°C).

The search for anti-nuclear antibodies was positive at a low titer. Paroxysmal nocturnal hemoglobinuria was excluded by cytofluorimetric tests. Bone marrow aspirate and biopsy showed an hyperplastic erythropoiesis, but ineffective. The patient initially received erythrocyte transfusions, but the increase of the Hb level was scanty and transient. On the basis of these data, a diagnosis of Coombs-negative AIHA with reticulocytopenia was made. The patient was initially treated with methylprednisolone (2 mg/kg/day), but after 2 weeks the hematologic picture was not changed. Subsequently, she received a course of immunosuppressive treatment (cyclophosphamide, 0.5 g/day for 5 days), while continuing steroids (40 mg/day), and transfusions. After one month she was admitted to our Institution. Hemoglobin concentration was 7.2 g/dL, MCV was 92.4 mm3 without reticulocytosis (2.7%). Laboratory signs of hemolysis were still present (indirect bilirubin 1.19 mg/dL, aptoglobin: 36 mg/dL). The bone marrow aspirate was compatible with hemolytic anemia. From January 2001 we administrated Cs-A (5 mg/kg/day), while continuing intermediate-dose steroids. After 6 weeks, a significant improvement was evident: Hb was 9.6 g/dL (without transfusions), MCV was 99.7 mm3, with 14.5% of reticulocytes. From then on, in spite of the tapering of steroids, the increase of Hb level continued, reaching a normal value (without hemolysis) after 14 weeks. Corticosteroids were definitely stopped after 9 months, and she is still in hematologic remission, while on Cs-A maintenance treatment (4 mg/kg/day).

PU370
A COMPUTERIZED CLINICAL AND LABORATORY DATABASE FOR THE STUDY OF G6PD DEFICIENCY
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The development of computer based systems to capture, store and elaborate clinical data is an important tool to improve the patient care and to enhance medical decision-making. This is especially true for genetic diseases showing heterogeneous clinical and laboratory features. G6PD deficiency is a widespread sex-linked hereditary enzymatic disease in humans with a marked molecular heterogeneity. The clinical manifestations range from hemolytic anemia, induced by drugs or infections, to favism, neonatal jaundice or, in a few sporadic cases, congenital nonspherocytic hemolytic anemia. The laboratory diagnosis relies on the demonstration of decreased G6PD activity in the red blood cells. In the heterozygous state, the residual enzyme activity is highly variable and a number of cases can escape the correct diagnosis. Therefore the definitive diagnosis is achieved by a diagnostic protocol including family study and both enzymatic and molecular tests. The prevention of acute hemolytic episodes is achieved by avoiding risk factor (i.e. fava beans, drugs), and taking into account the case history. We present a clinical and laboratory database, with integrated modules, which could be used as case sheet of G6PD deficient patients. The modules are designed according to logical criteria which are the bases of a standardized protocol for diagnosis and clinical follow up. The database consists of four sections containing homogeneous information: i) family and personal data, ii) clinical data, iii) laboratory findings, iv) follow-up data and information. To standardize data collection and avoid redundancy each section is arranged as a check-list. Each section includes a number of modules. The clinical data section includes four modules: i) family and pathological anamnesis, ii) clinical examination, iii) therapy and iv) follow up. The laboratory find-
ings are recorded in tree modules: i) basic hematologic and serological data; ii) biochemical data (red blood cell G6PD, PK, GSH), iii) molecular data. The software was developed in MS Access 2000 as application client-server with remote access by means of an internet browser. Security was implemented through insertion of a couple username/password. The user interface has been carefully designed to provide a user friendly tool, maintaining, at the same time, high-level interaction. Further facilities were added to the programme: the possibility to perform simple statistical elaboration, data export in various formats, production of printed reports of the case history. Our database model is useful to study the relationship between clinical features and biochemical or molecular data. At the same time the play back data consultation enables to make a patient related risk assessment. Such an exhaustive record of information could be used for case registration at national or international level and for epidemiological research.

PU371
ACANTHOCYTOSIS AND NEURODEGENERATIVE SYNDROMES: A NEW ABNORMALITY OF ERITROCYTE MEMBRANE PROTEINS
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Background: Most forms of acanthocytosis are associated with acquired or inherited abnormalities of membrane lipids. In rare subjects with acanthocytosis, membrane protein abnormalities have been detected but the mechanism whereby they lead to acanthocyte formation is unknown.1 Neuroacanthocytosis (NA) is a rare multisystem degenerative disorder of the nervous system associated with acanthocytosis in peripheral blood and normal lipoproteins. Mutations in the NA gene on chromosome 9q21 encoding chorein were recently found to cause the disease.2 Recently the chorein gene was identified and different mutations of chorein gene have been demonstrated in chorea-acanthocytosis.2 In this study we describe four patients with neuroacanthocytosis without chorein gene mutations and with a new specific alteration of erythrocyte membrane: a defect of protein 4.1R. Erythroid protein 4.1R is an 80-kDa skeletal protein necessary for structural organization and stability of red cell cytoskeleton. A protein 4.1R deficiency could give reason of the morphological abnormalities observed in these syndromes and a role of 4.1R in brain could be hypothesised.

Bone Marrow Aplasias
PU372
SEVERE T-MEDIATED BONE MARROW APLASIA IN A PATIENT WITH SPLENIC LYMPHOMA WITH VILLOUS LYMPHOCYTES (SLVL) PREVIOUSLY TREATED WITH FLUDARABINE REGIMEN
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A 70-year old man was sent to our hematology department because of a leukocytosis (wbc 30000/mm3) with lymphocytosis (20000/mm3) and mild anemia (Hb: 11.5 g/dL). At physical examination small nodes (size less than 1 cm) were present at all superficial sites. Moreover, it was found an increase of size (5 cm from left last rib) and of consistency of the spleen...
paralleled by a mild hepatomegaly. Total body CT scan confirmed the increase of spleen size without significant lymphohadenomegaly of deep lymphnodes. Morphology of peripheral circulating lymphoid cells showed that they were small size lymphoid cells with, sometimes, a small evident nucleolus and short cytoplasmic extroflessions (villi). Cytochemical analysis resulted negative for the tartrate resistant acid phosphatase (TRAP). The immunological pattern of lymphoid population was the following: CD19+, CD20+, CD22+, CD5± (expressed at low density only on a part of the B- lymphoid population), CD23-, CD43-, FM C7+, CD79b+, CD10-, CD11c-, CD103-, smig- . Bone marrow biopsy showed infiltration by an atypical CD20 lymphoid population formed by villous cells. On the basis of these data we diagnosed a splenic lymphoma with villous morphology. On the basis of these data we diagnosed a splenic lymphoma with villous morphology. Cytochemical analysis resulted negative for the tartrate resistant acid phosphatase (TRAP). The immunological pattern of lymphoid population was the following: CD19+, CD20+, CD22+, CD5± (expressed at low density only on a part of the B- lymphoid population), CD23-, CD43-, FM C7+, CD79b+, CD10-, CD11c-, CD103-, smig-. Bone marrow biopsy showed infiltration by an atypical CD20 lymphoid population formed by villous cells. On the basis of these data we diagnosed a splenic lymphoma with villous lymphocytes (SLVL). Therefore, the patient was subjected to the following treatment schedule: 25 mg/m2 fludarabine i.v. for 5 days every 4 weeks. Five total treatment cycles were performed and the treatment was well tolerated. One month after the last cycle the patient was subjected to a disease re-staging that showed only histological bone marrow infiltration by an atypical CD20 villous lymphoid population. Thereafter, the patient was subjected to a follow-up without therapy. After 6 months the patient developed a severe anemia (Hb: 6 g/dL), leukopenia (wbc: 1500/mmc) and thrombocytopenia (Plt: 60000/mmc). At the physical examination signs of disease recurrence were not present. Bone marrow examination showed a medullary hypoplasia with a minimal infiltration by an atypical CD20 villous lymphoid population. The molecular analysis with PCR of the marrow blood revealed the presence of the rearrangement of the γ gene of the T cell receptor (TCR). After 2 weeks of supportive care the clinical conditions of the patient worsened and the pancytopenia increased. A new bone marrow examination showed a severe aplasia with the absence of CD20 B lymphocyte infiltration but the presence of CD45R0 T lymphoid cells. The molecular analysis confirmed the presence of genetic clonal rearrangement of the γ gene of TCR. The patient died for infective complications before any specific treatment could be started. The development of autoimmune diseases or of second neoplasms in patients affected by B cell chronic lymphoproliferative disorders is well known, above all in the case of chronic lymphocytic leukemia. Until today, at our knowledge, T lymphoproliferative disorders able to induce a severe bone marrow aplasia were never described in patients affected by SLVL. Further studies are needed to investigate on the role of chemotherapy in the onset of the aplasia in patients affected by SLVL and on the real incidence in the population of the SLVL since the latter is often misdiagnosed and confused as a generic B cell chronic lymphoproliferative disease or as an atypical chronic lymphocytic leukemia.

**PU373**

**SUCCESSFUL TREATMENT OF ACQUIRED APLASTIC ANEMIA WITH RITUXIMAB IN A PATIENT WITH FOLLICULAR NHL**

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Acquired aplastic anemia (AA) is characterized by peripheral blood pancytopenia and hypocellular bone marrow. Idiopathic AA represents almost 90% of cases of acquired AA and the diagnosis of the disease is mainly one of exclusion, made by eliminating the other causes of marrow hypocellularity. The choice of primary treatment should be based on the availability of an HLA identical sibling, the age of the patient and the severity of the disease. Patients without a donor should be given immunosuppressive therapy (IS) as the first line therapy with ATG based regimen combined with cyclosporin A. Recently, few reports suggest a role of monoclonal anti-CD 20 antibody Rituximab in the treatment of monolinear autoimmune disease as pure red cell aplasia or immune thrombocytopenic purpura refractory to the conventional IS therapy. Herein we report a case of idiopathic AA treated with Rituximab at our Instute. A female patient, 64 years old, affected by follicular NHL, stage IIA, was admitted at EIO in February 1999. She received chlorambucil and prednisolone as first-line therapy, achieving a complete remission after 5 cycles. In April 2000 she had a disease recurrence in the abdomen and was treated with 4 cycles of Rituximab at standard dose, obtaining again a complete response. One year later, without any clinical or radiological evidence of disease progression, she developed a severe peripheral pancytopenia requiring intensive blood and platelet support. A bone marrow trephine biopsy and a marrow aspirate showed a cellularity <5% without evidence of lymphoproliferative disease or myelodysplastic syndromes, cytogenetic was normal (46,xx). Further evaluations excluded a paroxysmal nocturnal hemoglobinuria. We started a steroid therapy with full dose prednisolone that did not change the peripheral pancytopenia and the need for blood transfusions. Considering the recent reports describing the clinical activity of anti-CD20 in patients with autoimmune disorders, we decided to re-treat our patient with standard dose Rituximab (375 mg/m2). A prompt hematologic recovery was observed just after the first administration; the patient received a total of 4 weekly doses of Rituximab without any complications. The bone marrow re-evaluation performed one month after the end of the treatment showed a 40% cellularity with normal hematopoiesis. The patient is still in complete hematologic remission. This experience shows promising efficacy of Rituximab as single agent in acquired AA; further experience are need to confirm our single observation.
PU374
INCREASED TNF-α, IFN-γ AND APOPTOSIS IN A CASE OF PURE RED CELL APLASIA
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Pure red cell aplasia (PRCA) is associated with viral infection (especially parvovirus B19), autoimmune, and lymphoproliferative diseases. Antibodies cytotoxic for marrow erythroid cells or directed against erythropoietin have been described in this setting and T cells in PRCA patients may suppress erythropoiesis in vitro. In a case of PRCA associated with clonal expansion of granular lymphocytes, the γ8 T-cells lysed bone marrow proerythroblasts. Finally, PRCA responds to immunosuppressive therapies. An autoimmune pathogenesis has been hypothesized also in aplastic anemia (AA), a disease closely related to PRCA. Increased Th-1 cytokines (IL-2, TNF-α, IFN-γ and MIP-1α) have been demonstrated in AA, and stem cells presented increased apoptosis. In a PRCA patient, we have studied cytokine production by peripheral blood (PB) and bone marrow (BM) mononuclear cells (MC), and explored apoptosis in PBMC and BMMC. SA, a 65 years old male, presented severe anemia (66 g/L), and a diagnosis of PRCA was made by bone marrow aspirate and trephine biopsy. The patient was treated with antilymphocyte globulin, cyclosporine and steroids without obtaining clinical response. We evaluated TNF-α, IFN-γ, and IL-2 in the peripheral blood (PB) and bone marrow (BM) mononuclear cells (MC), and apoptosis in the PBMC and BM MC, and short term cultures of in toto BM, and CD34+ BM MC. TNF-α production by PMA stimulated patient’s PBMC was more than 10 times that observed in control cultures. On the contrary, both IL-2 and IFN-gamma production were reduced in patient’s cultures compared with controls. Considering cytokine production by BM MC cultures, IFN-γ was 10 times greater in the patient than in control, IL-2 was comparable, and TNF-α reduced. As far as DNA-fragmentation is concerned, patient’s PBMC showed increased percentages compared with healthy controls. Values were even higher in patient’s BM than in PB. Addition of TNF-α further increased DNA fragmentation, particularly in BM MC. Short term colony-forming unit assays showed no significant differences in the clonogenic activity of CD 34+ versus BM MC, and of BM MC versus BM MM incubated with autologous or human AB serum, with or without Campath-1H. In particular, in all the cases BFU-E were absent, while CFU-GM were in the normal range. Our results showed that in this PRCA patient there was an increased production of TNF-α and IFN-γ by PBMC and BM MC, respectively. Both cytokines are mediators of Th-1 and cytotoxic T-cell responses and thus their increase may play a role in cellular immune-mediated destruction of hematopoietic stem cells. IFN-γ overproduction in bone marrow but not in peripheral blood is interesting, suggesting a compartmentalization of the cytokotic response in the site of erythropoiesis. Furthermore, in PBMC and, more markedly, in patient’s bone marrow, we demonstrated an increased apoptosis, that was even greater in the presence of TNF-α. These data are consistent with the hypothesis that depletion of stem cells occurs through increased apoptosis, as already reported in AA. Finally, results of bone marrow clonogenic activity are consistent with selective destruction of early erythroid progenitors. In conclusion, our results demonstrate an increased Th-1 cytokine response and an increased apoptosis, suggesting their role in the destruction of erythroid progenitors in a case of PRCA.

PU375
PANCYTOPENIA ASSOCIATED WITH LEFLUNOMIDE AND METHOTREXATE (MTX): REPORT OF ONE CASE
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Leflunomide is a novel agent for the treatment of rheumatoid arthritis. It is used in cases resistant to other therapies and its immunsuppressive effect is associated to an antiproliferative action. Recently, some australian authors reported about 9 cases of pancytopenia (hemoglobin <10 g/dL, platelets <100×10^9/mm³, neutrophils<1.5×10^9/mm³) occurring in patients taking leflunomide. Seven out of 9 patients were taking concomitant MTX (Ann Pharmacother 2003;37:149). Prior to this report there was only one published case of pancytopenia associated with leflunomide treatment. Also MTX showed a pancytopenia rate of 2% when assumed for rheumatoid arthritis at doses of 7.5-12.5 mg/week. We hereby report a well documented case of pancytopenia in a 73 years old woman who was taking MTX since 4 years. Leflunomide was introduced a month before, and stopped two weeks later when fever appeared. Leflunomide was suspended since 14 days when the patient was admitted in our department. She had fever up to 39,5°C, diffuse bilateral rales, poor general conditions, mild dyspnea and hypossiemia (PO2 52 mmHg). Blood and urine cultures were negative. Chest X ray did not show broncho-pneumonic infiltrates. Neutrophils were 600/mm³, hemoglobin 7.7 g/dl, platelets 48.000/mm³, M CV was 103 mm³. Bone marrow aspirate showed global hypoplasia; erytroid series was well represented with megaloblastic features; myelod series was
Aplastic anemia (AA) is a rare disorder of hemopoiesis characterized by bone marrow failure due to a variety of causes (genetic abnormalities; acquired factors as radiation, drugs, chemicals, viruses, immunological diseases, idopathic). The pathophysiology of aplasia is largely unknown, but many evidences suggest an autoimmune mechanism; indeed, immunosuppression represents the golden standard therapy for AA, with a high rate of success. We report a rare case of aplastic anemia associated with two episodes of eosinophilic gastroenteritis. A 26-years old female was observed three years ago for acute gastroenteritis with severe abdominal pain, ascites and marked eosinophilia. In the mean time, peripheral blood counts spontaneously tended to normalize (Hb 11 g/dL without transfusional support, ANC 4,000/mmc, plt. 70,000/mm³). Gastric and colonic endoscopy showed protuberances of the gut wall by diffuse eosinophilic infiltration, and ascitic cytology showed marked eosinophilia. Bone istology revealed a marrow of rich cellularity with marked eosinophilic infiltration. Cytogenetic analysis showed a normal female karyotype. Considering the recent good results of imatinib in hypereosinophilic syndrome (HES), we administered STI 571 at the dose of 200 mg/day for seven days, but the clinical condition worsened and eosinophils increased. The patient was then treated with prednisolone 1 mg/kg/day with prompt resolution of symptoms and eosinophil normalization. However, peripheral blood counts fell again and she returned aplastic (Hb < 5 g/dL, ANC < 500/mmc, plt. < 10,000/mm³). The patient is now receiving only supportive treatment. Since a HLA-identical sibling was not available, unrelated donor search has been activated; in the mean time, the patient will receive a second ALG course. This is a unique case of aplastic anemia with recurrent eosinophilic gastroenteritis, which was not responsive to STI 571. We are puzzled from the association between these two extremely rare diseases, and by the spontaneous recovery from AA when eosinophilic gastroenteritis recurred.

Cytogenetics and Molecular Genetics

PU377
EVALUATION OF TELOMERASE ACTIVITY IN PATIENTS WITH ACUTE LEUKEMIAS AND MYELODISPLASTIC SYNDROMES
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Human telomeres are specialized chromosomal and structures composed of TTAGGG repeats. They protect chromosomes from degradation, fusion and recombi-
nation. However, in normal cells, the number of cell division is limited and the main reason for that is the progressive loss of telomeric sequences. This process leads also to cell senescence when a critical telomere length is reached. The complete replication of telomeric sequences at the termini of eukaryotic chromosomes requires a special enzyme complex, telomerase, an RNA-dependent DNA polymerase, which is not present in most somatic cells (only human primitive hematopoietic cells and activated lymphocytes have borderline telomerase activity). In contrast, immortalized cell lines and the majority of malignant tumors demonstrate high telomerase activity, stable telomere length and unlimited proliferative potential. We analysed the telomerase activity in 39 AML and 28 MDS patients. Moreover 26 normal subjects were evaluated. Methods. Fluorescence TRAP assay was performed on bone marrow samples as previously described (1) and expressed in arbitrary enzymatic units (AEU). Results. Twenty-six pts with AML were evaluated at diagnosis. Their median telomerase value was 261.5 AEU (extr. 51-2723). Six pts were evaluated after therapy. Their median value was 64.5 AEU (extr. 56-126). The 18 pts with M DS had a median telomerase value of 95.5 AEU (extr. 53-2458). Normal controls showed a median value of 94.0 AEU (extr. 46-346). The correlation of these data with other prognostic parameters is still ongoing. These data confirm previous data showing a marked increase in telomerase activity in AML with a reduction within normal range after therapy. Differently from other studies, the median values of M DS pts and normal individuals, in our study appear identical.

References

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PU378
ANALYSIS OF THE DIHYDROFOLATE REDUCTASE GENE IN ACUTE LEUKEMIA BLASTS BY FLUORESCENCE IN SITU HYBRIDIZATION
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The key role of the dihydrofolate reductase (DHFR) system is related to its involvement in the metabolism of nucleic acids. Methotrexate (MTX) is a DHFR inhibitor and it is used very often in a clinical setting as anti-cancer drug. Biochemical and molecular studies showed a correlation among level of DHFR activity, gene amplification and resistance to MTX in experimental systems, but only few studies have been performed in human cells from patients with acute leukemia (AL). Since an increased level of DHFR activity is detectable in blasts from leukemia patients at the onset of the disease or at relapse by histochemical methods, it can be argued that overexpression of the DHFR gene may be involved in the development of clinical resistance to MTX. In order to investigate the relation between increased DHFR level and gene amplification we performed cytochemical and molecular studies in PHA-stimulated peripheral blood lymphocytes from 8 normal donors, in MTX resistant HeLa cells and in bone marrow blasts from 16 patients with AL, classified as follows: 11 cases of acute myeloid leukemia (AML) (3 M 1, 3 M 2, 2 M 3 and 3 M 5), 9 at the onset and 2 at relapse, and 5 cases of acute lymphoid leukemia (ALL), all of the common type, 3 at the onset and 2 at relapse. The DHFR cytochemical reaction was performed according to Gerzeli and De Piceis Polver and the optical density (OD) of the reaction product was quantified employing a Vickers M 86 scanning and integrating microdensitometer. A plasmide containing the DHFR gene labeled with biotin was utilized as a probe for in situ hybridisation. The fluorescence signal (FS) was quantified using the Scion image software program. Significantly higher levels of enzyme activity were observed in lymphoblasts (mean 65±10.6) than in normal lymphocytes (mean 38.4±2.6) (p=0.004) and in AML blasts (mean 167.6±12.6) than in lymphoblasts (p=0.00001). The strongest level of enzyme activity was observed in MTX resistant HeLa cells carrying DHFR gene amplification (mean 324.9±11). In AL an heterogeneous hybridization pattern was observed at the single cell level. However, the FS with the DHFR cDNA probe in interphase nuclei was about tenfold higher in lymphoblasts than in normal lymphocytes and about hundredfold higher in myeloblasts than in lymphoblasts. Relapsed cases showed the highest fluorescence intensity signal. These findings suggest an amplification of the DHFR gene in all our AML cases both at the onset and in relapse; also ALL cases showed a slight amplification of the DHFR gene. Therefore, the increased expression of DHFR in leukemic blasts is due to gene amplification. The high levels in AML might explain the MTX natural resistance of myeloblasts; lymphoblasts with increased levels of DHFR may present a reduced sensitivity to the drug. A thorough follow-up of our patients could solve this interesting problem.
PH NEGATIVE CLONES IN CML PATIENTS IN COMPLETE CYTOGENETIC REMISSION AFTER THERAPY WITH IMATINIB MESYLATE: A MOLECULAR CYTOGENETIC STUDY

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While clonal evolution with secondary cytogenetics abnormalities additional to Ph positive clones are seen in up to 60-80% of CML patients that progresses to advanced stages of the disease, cases with additional Ph negative clones have been only rarely reported with interferon therapy. This seems to be more frequent in patients treated with imatinib than during other therapy regimens. Recently, Andersen et al. reviewed 17 patients from the literature who developed a Ph negative unrelated clone after therapy with imatinib (8 pts) or other therapies (9 pts) including interferon, busulfan, hydroxurea and busulfan-melphalan with autologous bone marrow transplantation. In particular, 11 out of 17 patients had a trisomy 8, 2 of these cases also had a monosomy 7 or del(7q), while other abnormalities involved chromosomes 5q, 13q, 11q. Interestingly, 4 out of 17 patients, 2 of them with a +8, 1 with a +8 and 7, 1 with a del(7q), developed acute myeloid leukemia (AML) or myelodysplasia (MDS). We had 5 of 29 CML patients (17.2%) treated at our Institution who after imatinib mesylate developed novel Ph negative clones in complete cytogenetic remission (CCR) after a mean time of 11 months (range 6-28) (Table 1).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Prior therapy</th>
<th>New clone</th>
<th>Months from STI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>F</td>
<td>HU (4)</td>
<td>+ 8</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>F</td>
<td>HU (2); ABMT</td>
<td>+ 8</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>M</td>
<td>—</td>
<td>+ 8</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>M</td>
<td>HU (6)</td>
<td>t(6;7)</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>F</td>
<td>—</td>
<td>dup(1q)</td>
<td>6</td>
</tr>
</tbody>
</table>

Three of the 5 patients had a +8 in 1 to 13 cells, one patient developed a novel t(6;7)(p24;q21), the last case a dup(1)(q11q21). All patients were previously treated with HU or IFN except for one. We didn’t see any sign of MDS. Patient n 5 had a full disappearance of the Ph+ cells, maintaining the dup(1q) as the sole aberration after 6 months of therapy. A fluorescence in situ hybridization (FISH) study of the diagnosis samples in patients #1, 2 and 3 utilising a CEP 8 probe (Vysis, Downers Grove, IL, USA showed a mean of 3% (2-4) of cells with trisomy 8, under a normal cut off value. All patients are alive, in good clinical conditions, maintaining hematologic and CCR. It is unclear at the moment the meaning and the mechanism of emergence of additional clonal abnormalities: it can be suggested that Ph+ cells have a proliferative advantage at diagnosis on other clonal cells and that imatinib discloses other leukemia triggers, although mechanisms of resistance or mutagenesis cannot be ruled out.
and cluster families. We investigated rearrangements of BCL2 (MBR and mcr) and BCL1 genes and the presence of clonal rearrangements of the immunoglobulin heavy chain (VD-JH) by PCR and the presence of Bcr/Ab1 translocations (p210 and p190) by RT-PCR. Amplification of the $\beta$ globin gene and the normal Ab1 allele was used to test the quality of the template DNA and cDNA, respectively. Amplification of normal Ab1 gene was also used as an external control for the RT-PCR. None of the samples analyzed showed evidence of Bcr/Ab1, BCL2 MBR or BCL1 gene rearrangements. As compared to the control group, one sample in the familial cluster group showed a translocation involving BCL2 mcr but this finding was not statistically significant. The same sample was also positive for the clonal rearrangement of the immunoglobulin heavy chain, consisting with a diagnosis of follicular lymphoma. These findings do not support the hypothesis of a higher frequency of specific gene rearrangements in familial clusters of lymphoproliferative disorders. We are currently analyzing immuno-fluorescent parameters as well as rearrangements of the T-cell receptor (TCR) in these groups.

**PU381**

**A CASE OF ADULT ACUTE MYELOID LEUKEMIA (M5A) WITH A NEAR-TETRAPLOID KARYOTYPE CHARACTERIZED BY MONOSOMY 5 AND 16**

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While hyperdiploidy is frequently observed in acute lymphoblastic leukemia, tetraploidy is rarely observed in acute myeloblastic leukemia (AML) in which gains and losses of whole chromosomes occur frequently, usually at diagnosis. A 76-year-old man was admitted to our Division with dyspnea and weakness. A peripheral blood cell count showed: WBC 29.6×10^6/mL with 90% of blast cells, Hb 9.4 g/dL, PLT 55×10^6/mL. Physical examination was normal except for liver enlargement. Bone marrow examination confirmed a diagnosis of AML M5a with 90% of blast cells. Leukemic cells showed positivity for CD13, CD33, CD34, CD117, MP07, high intensity of BCL2. Bone marrow cells from unstimulated 24-48 h cultures in RPMI 1640 20% FCS were harvested for cytogenetic analysis and slides were prepared for examination. At least 20 metaphases, G-banded by Wright’s stain, were examined and the ISCN nomenclature was used for the description of chromosomal abnormalities. The karyotype was: 46,XY [1]; 92, XXY [1]; 90, XXY [5], 5.41 [18]. The patient was treated with daunorubicin (DNR) 70 mg/day for 3 days, and cytosine arabinoside (AraC) 200 mg/day for 7 days achieving a morphological and cytogenetic complete remission. Twenty cases of tetraploidy in AML are reported in the Mitelman database: 8 females, 12 males, most above 65 years old. FAB subtypes were: M0 (1), M1 (2), M2 (4), M3 (2), M4 (2), M5 (1), M7 (1), NOS(7). Interestingly all cases of M2 reported t(8;21) as an additional abnormality. According to previous reports the prognosis seems poor, and only two patients achieved a complete remission after the first cycle of chemotherapy. To our knowledge this is the second case of AML M5 reported in the literature showing a tetraploid karyotype by standard cytogenetic analysis. Despite monosomy 5 present in the tetraploid clone, the patient achieved a complete remission and is actually in a good clinical condition.

**PU382**

**MOLECULAR HETEROGENEITY OF ACUTE LEUKEMIAS WITH REARRANGEMENT OF THE 11Q23 LOCUS**


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Structural abnormalities of the 11q23 band with MLL gene translocation is a recurrent chromosome change in leukaemia and a recent proposal by WHO specifies a separate category for AL with 11q23+/MLL+. Conversely the outcome of patients with 11q23+ is not well defined: this possibly reflects the marked heterogeneity of 11q23 aberrations. As combining CC and molecular methods may reveal discrepancy between 11q23+ and MLL+ cases (Ibrahim et al., 2000) the definition of diagnostic methods may have clinical implications. Three-hundred and thirty newly diagnosed AL patients were analyzed with CC and FISH using a commercial probe for the 11q23 locus (Vysis Inc.) that should detect all MLL translocations. Leukemia was diagnosed between January 1992 and November 2002 and classified according to the FAB criteria: 290=AML, 44=ALL, and 3=biphenotypic AL, median age=48 years (range 5-81 years). Samples were processed following standard cytogenetic procedures after direct and synchronized short term cultures. Karyotypes were classified according to the ISCN 1995 Nomenclature. FISH was carried out on cytogenetic pellets (stored at -20°C in Carnoy’s solution) following established protocols (von Bergh et al. 2000). In 15 patients, who were 11q23-/MLL-, also Multi-Colour-FISH karyotyping (M-FISH) was done (Spectra-Vysion, Vysis Inc.). CC showed clonal aberrations in 44.5% (n=147/330), normal karyotype in 40% (n=130/330) and failed in 16% (n=53/330). 11q23 rearrangement was observed in 7.2% of cases with assessable CC (n=20/277). FISH detected MLL
rearrangement in 5% (n=17/330): 15 translocations (2 missed by CC because of failure) 1 amplification and 1 deletion. M-FISH identified a cryptic t(9;11)(p21;q23); t(11;16)(q23;p13); t(11;15)(q23;q12); t(11;12) (q23-24;q24); add(11)(q23); ish. t(9;11) (p21;q23-24). Following literature search we found a few comparable studies which report an incidence of 11q23-/MLL+ (detected by FISH) between 7% and 22%.

Overall FISH, CC and M-FISH detected 11q23+/MLL+ cases were not found, but the combining of CC and FISH allowed to define better MLL involvement in 6 patients (26%), while in an other 6 patients of this group, FISH ruled out MLL gene rearrangement (i.e.: t(2;11) (p21;q23); t(11;16)(q23;p13); t(11;15)(q23;q12); t(11;12) (q23-24;q24); add(11)(q23); ish. t(9;11) (p21;q23-24)).

PH+ CML treated with interferon-alfa (α-IFN) or Imatinib. We report 2 cases of Ph+ CML patients, persisting during Imatinib therapy. The patient is well and in Ph cytogenetic remission. The second case is a Ph+ CML patient who, after about 6 years of IFN therapy showed the following karyotype: 46,XY, t(9;22)(q34;q11), leukemia. The clinical significance of these new Ph- clones is unclear and has to be evaluated in further studies. These changes can be due to the effect of therapy or the selective Ph suppression obtained with therapy may allow the evolution and the expansion of other suppressed clones existing simultaneously or arising in time. Funding: this work was supported by grant from: Cofin 2002, Ateneo 60%, Fondazione del Monte di Bologna e di Ravenna.

PU383

EMERGENCE OF CLONAL CYTOGENETIC ABNORMALITIES IN PH- CELLS DURING TREATMENT WITH α-IFN AND IMATINIB IN PH+ CML PATIENTS


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Chronic myeloid leukemia (CML) is characterized by the reciprocal translocation t(9;22)(q34;q11), leading to the formation of a BCR/ABL fusion gene which results in constitutive activation of the ABL tyrosine kinase. Therapies with α-IFN and more recently Imatinib are able to give hematologic and cytogenetic remission (CR). Recent reports focused on emergence of new clonal abnormalities in Ph- cells in patient with Ph+ CML treated with α-IFN or Imatinib. We report 2 cases of Ph+ CML who, after α-IFN therapy, showed Ph- clones, persisting during Imatinib therapy. A 61 years-old male was diagnosed to have CML in June 1997. At diagnosis, cytogenetic analyses showed only 434 cells with Ph rearrangement were found. In addition, FISH with a LSI MLL Dual Color probe (Vysis, Inc.) in order to evaluate MLL rearrangement was negative. RT/PCR for BCR/ABL was weakly positive and quantitative PCR has been performed during the course of therapy. The patient is well and in Ph cytogenetic remission. The second case is a Ph+ CML patient who, after about 6 years of IFN therapy showed the following karyotype: 46,XY, del(11)(q14q23) (6/50). In this case, the abnormality was transient because the patient didn't show these abnormalities in the following studies. Then, he was enrolled in the protocol of therapy with Imatinib. After 3 months he reached complete CR. The same abnormality involving 11q appeared after one year of Imatinib therapy, in only 1 on 50 observed metaphases.

PU384

GENOMIC ANATOMY OF RECIPROCAL TRANSLOCATION T(11;20) AND MOLECULAR CHARACTERIZATION OF BOTH FUSION TRANSCRIPTS


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The TOP1 gene localized to chromosome band 20q11, encodes a monomeric protein of 765 aa, the human topoisomerase I. Topoisomerase I (top1) promotes the relaxation of DNA superhelical tension by introducing transient single-stranded break in duplex DNA and it is vital for the processes of replication, transcription and...
recombination. Topol consists of four major regions: the NH2-terminal, core, linker and COOH-terminal domains. The TOP1 gene was first reported as a fusion gene with nucleoporin gene NUP98 located on 11p15, in two therapy-related myelodysplastic syndrome (t-MDS) patients with the t(11;20)(p15;q11) by Ahuja et al. The NUP98 gene product is a 98 Kd component of the nuclear pore complex (NPC), which regulates the nucleocytoplasmic transport of protein and RNA. As yet, of the two possible transcript products resulting in the translocation, only the der(11) product has been identified. The latter contains the FXFG repeats of NUP98 fused to the majority of TOP1, including its catalytic domain. We have investigated and characterized NUP98/TOP1 gene fusion in a de novo LMA-M2 with t(11;20)(p15;q11), identified in G-band and confirmed by FISH. The RT-PCR studies performed on the total RNA of bone marrow leukemic cells detected the NUP98-TOP1 transcript and the reciprocal TOP1-NUP98 der(20) transcript, never found before in other cases. We have also characterized the genomic breakpoints on der(11) and der(20). The sequence analysis showed that the breakpoint is different from the other genomically characterised t(11;20), close to LINE2 of NUP98 intron13 and upstream of a MIR interspersed repetitive element of TOP1 intron 7. Previous studies suggest a possible susceptibility of these introns to chemotherapeutic induced breakage. Note, however, that our patient has not been treated with multiagent chemotherapy regimens and that both fusion transcripts find almost equal expression in the leukemic cells.

**PU385**

ELEVATED EXPRESSION OF PGP PROTEIN AND ABNORMALITIES OF CHROMOSOME 7

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Multidrug resistance (MDR) is the major obstacle to treatment of acute leukemias. Although many mechanisms are known as implicated in MDR, the most important is the overexpression of P-glycoprotein (Pgp), a product of MDR-1 gene located at chromosome 7q21. Pgp is a membrane glycoprotein of 170 kilodalton with the function of ATP-dependent efflux pump able to lower the cellular concentration of most widely used anti-cancer lyphic drugs, such as Vinca alkaloids, epipodophiltotoxins, anthracyclines. Pgp overexpression impairs the intracytoplasmatic accumulation of drugs, thus allowing cell survival. A correlation of long arm of chromosome 7 abnormalities with overexpression of the MDR1 gene has been reported in cell lines but rarely documented in acute leukemia patients. We report three cases of acute leukemia with chromosome 7 abnormalities and overexpression of Pgp. The first patient received diagnosis of AM L-5 M5 CD13+, CD33+, CD11b+, CD11a+, CD36+ and HLA-DR+. Cytogenetic analysys showed the following karyotype: 47 XY +der7. The patient was resistant to EORTC AM L 10 protocol and died a few months later. The second patient received diagnosis of γδ T cell leukemia/lymphoma. Cytogenetic analyses showed a complex karyotype: 46XY/ 47 XY+ i(7q)/ 48 XY +i(7q)+8. He was submitted to different therapy lines without efficacy, and died a few months later of disease progression. The third patient received diagnosis of AML M1 CD13+, CD33+, CD117+, CD11b+, CD34+, HLA-DR+; the karyotype was 46 XY i(7q). The patient started the AM L 12 EORTC/GIMEMA protocol without effect and now is receiving a second line treatment. In all three patients we were able to detect at diagnosis a high level of Pgp on cell surface by immunophenotype using the MRK16 Mo Ab. The presence in all patients of chromosome 7 abnormalities, Pgp overexpression and resistance to chemotherapy suggest the possibility of MDR gene amplification. Cytogenetic and molecular studies are ongoing in order to assess if Pgp overexpression in these resistant leukemias is due to the presence of multiple copies of MDR1 gene or to disregulation of its expression.

**PU386**

13Q14 DELETION DETECTED BY DUAL COLOR FISH ON IMMUNO-SELECTED PLASMA CELLS

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Abnormalities involving chr.13 are a critical event in MM and are predictive for short survival after conventional dose and high dose chemotherapy. FISH studies have revealed that 13q14 deletions define a distinct entity of MM with unique biological and clinical features and mark the transformation from MGUS to MM. So far it has been recommended routine FISH 13 deletion assessment for patients considered for HDT and MGUS/MM patients. We investigated bone marrow (BM) specimen from 5 MM and 4 MGUS patients. To test for 13q del we used the DNA probe D13S272, whose effectiveness was demonstrated in other studies. Hybridizations were performed on cytospin slides of BM PCs positively selected by means of anti-CD138-coated magnetic beads; we used a dual color FISH assays combining D13S272, with a probe specific for the centromeric region of chr.10, to control for hybridization efficiency. The cut-off value was 8.7. To further improve our specificity we decided to consider
patients as positive for the deletions only if they had over 10% abnormal cells. Significant percentages of cells with one signal were found in three MM patients and in one MGUS patient. According to recent observations, FISH detects -13/13q- in 21-45% of MGUS patients, 33-53% of patients with newly diagnosed MM and in up to 73% of patients with relapsed MM. Therefore FISH has been proposed as the first choice for analysis and diagnostic work-up of MGUS and MM pts. But the results may be hampered by the limited number of PCs in MGUS and by the loss of hybridization signals due to insufficient hybridization conditions. To resolve this issue we performed a dual color FISH on PCs purified using anti CD138 magnetic beads; the PCs recovery is high (85%) so this assay is suitable in MGUS, then is specific because with the reference probe we control the hybridization efficiency and is faster because FISH is performed on cytoprep avoiding culture time. A sensitive and unequivocal detection of 13q rearrangement is of great clinical importance. We consider FISH assessment of 13q deletion in dual color hybridization, with PC positive selection, a reliable diagnostic method.

PU386bis
EVIDENCE OF BREAKAGE-FUSION-BRIDGE CYCLES IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA
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The Breakage-Fusion-Bridge cycle (BBF) is one of the mechanisms of genetic variability consisting in a sequence of chromosome rupture - sister chromatid reunion - dicentric chromosome formation which, in turn, breaks again at anaphase triggering a further BBF. This mechanism has been reported in human solid tumors showing chromosomal aberrations, including dicentric chromosomes, ring chromosomes, and telomeric associations. BBF cycles can produce intra-chromosomal gene amplification via a double-strand break distal to the amplified gene and they may be mediated by chromosomal fragile sites (CFS). We present data supporting BBF occurrence in an adult acute lymphoblastic leukemia (ALL) case. The phenomenon involves chromosome 1, seems to be mediated by CFS activation, and leads to COAS3 gene amplification. Partial chromosome paints (PCPs) for chromosome 1p and 1q were DOP-amplified and used as probes for fluorescence in situ hybridization (FISH). Plasmid probe pZ5.1 contains centromeric sequences specific for chromosomes 1, 5 and 19. To assess a role for CFS in oncogene intrachromosomal amplification we selected BAC clones RPCI-11 534L20 and RPCI-11 763B22 specific for COAS3 and MDM4 oncogenes mapping in 1q21.2 and 1q32.1, respectively. RPCI-11 332H17 in 1q24.2 was used for breakage characterization. University of California Santa Cruz database was queried for probe location. Conventional cytogenetic analysis revealed the presence of the following karyotype: 46,XX, dup(1)(q21;q32), t(8;14)(q24;q32). The cohybridization experiment using PCP#1p, PCP#1q and pZ5.1 showed the presence of 13 cellular clones characterized by different chromosome 1 rearrangements. Among them, two clones were prevalent (48.6% and 19.1%, respectively): the former showed a heterochromatic region apart from the centromere, whereas the latter was characterized by an unusually long 1q arm. Moreover, a dicentric chromosome 1 was present in 12.1% of cells. FISH cohybridization using RPCI-11 534L20 and RPCI-11 763B22 showed COAS3 oncogene amplification in all metaphases, whereas additional copies of the MDM4 oncogene were observed in fewer cell numbers. In some cases, the cohybridization pattern revealed that the region included between these genes appears as part of a large inverted duplication, strongly suggesting a BBF cycle model. Detailed molecular characterization of the breakage clearly visible in a clone was performed. The use of BAC/PAC clones, mapping in the region delimited by the COAS3 and MDM4 oncogenes, allowed us to restrict the break inside RPCI-11 332H17. The use of appropriate molecular cytogenetic probes allowed us to detect different cellular clones showing chromosome 1 instability. The meaning of the oncogenes extra-copies remains to be explained, although the poor outcome of this case could be related to these data. CFS have been recently hypothesized to trigger the BBF phenomenon in cancer. Thus, activation of several chromosome 1 CFS could be hypothesized in our case to explain the clonal diversity observed. Moreover, it was possible to perform a detailed molecular cytogenetic characterization of a clone breakage that suggested the involvement of FRA1G located in 1q24. The present study highlights for the first time that BBF cycles could represent a mechanism for genomic instability in leukemia, generating a heterogeneous pattern of structural chromosome aberrations.
Hemopoiesis, Cytokines and Stem Cells

PU387
COMPARISON OF SERUM LEPTIN LEVELS IN CRITICAL PATIENTS AND IN HEALTHY SUBJECTS AND ITS RELATIONSHIPS WITH BLOOD CELL PARAMETERS

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Leptin exhibits a functional pleiotropy mediated by leptin receptor (LR). Its primary physiologic role consists in controlling adipose tissue metabolism, but it also play a role in fetal and adult erythroid and myeloid functions, leptin could contribute, with other cytokines, to the clinical course of several hematopoietic disorders, particularly low-grade NHL. In particular, TNF-α and sCD23 levels have been correlated with disease stage in LG NHL and B-CLL. We have evaluated the serum levels of several cytokines (TNF-α, sCD23, IL-1β and IL-6) before and after therapy in LG and HG NHL and B-CLL at diagnosis and tried to correlate the results with clinical stage and response to treatment. Serum IL-1β, IL-6, TNF-α and sCD23 were measured employing a quantitative sandwich enzyme immuno assay technique (EIA). The minimum detectable doses were: <1 pg/mL for IL-1β; <1 pg/mL for IL-6; <2 pg/mL for TNF-α and <10.3 U/mL for sCD23. Results. 57 high grade NHL pts (24 I-I ist; 33 III-IV st.) were evaluated. Their median value for LDH was 354 U (extr.262-1926) and 2.00 mg/dL for β2 microglobulin (β2M) was 2.00. Median TNF-α values were 6.9 pg/mL (extr. 2.0-30.3) for I-II st.; 6.4 pg/mL (extr. 2.0-88.0) for st.III-IV. Twenty-five patients were evaluated after therapy. All the 11 responding pts showed a normalization (<2 pg/mL) of the TNF-α values. The SD pt showed a reduction from 18 to 4 pg/mL while the pt with PD had an increase from 26 to 59 pg/mL. sCD23 values were 64.4 U/mL (extr.18-300) for st. I-II; 98.4 U/mL (extr.19-400) for st. III-IV pts. After therapy median value was 42.3 (extr.10-133.5) U/mL. Reduction was observed in all responding pts. Same value (133.5 vs156) in the PD pt. 39 low grade NHL pts (8 st. I-II and 31 st. III-IV) were evaluated. Median TNF-α values were 3.1 pg/mL (extr.2.0-12.0) in st. I-II and 13.5 pg/mL (extr.2.0-49.4) in st. III-IV pts. After therapy median value was 42.3 (extr.10-133.5) U/mL. Reduction was observed in all responding pts. sCD23 was 65.8 U/mL (extr.35.0-400.0) in st. I-II and 400.0 (extr.10.0-1600.0) in st. III-IV. After therapy, median value was 47.4 (extr.10.0-503.6) U/mL. SD pt had a reduction from 1600.0 to 303.6 U/mL, while the PD pt had a variation from 98.9 to 66.3 U/mL. IL-1β and IL-6 were undetectable in the normal range, respectively, in all the patients. These data confirm previous results in low grade NHL and show that also in HG NHL the levels of TNF-α and sCD23 may be of prognostic importance.

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CD69 is expressed on stem cells and therefore not appropriate to detect minimal residual disease in acute leukemia

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Objective: Recently CD69 was described as an aberrant marker for acute myeloid leukemia cells. The aim of this study was to verify the suitability of CD69 in evaluation of minimal residual disease. Methods: Peripheral blood stem cell products (PBSCP) from 14 patients (10 multiple myeloma, two acute myeloid leukemia, one non Hodgkin lymphoma and one chronic myeloid leukemia) collected after administration of mobilizing chemotherapy and G-CSF, 3 healthy stem cell donors stimulated only with G-CSF and 22 unstimulated bone marrows were analysed. Expression of CD69 PE and CD34 PerCP was analysed using a flow cytometer (FACSCalibur BD Biosciences) with a gate on the fluoresence 3 detector to ensure that at least 5000 CD34+ cells were analysed in each sample. Results. A median of 53% (range 29-72%) of CD34+ stem cells from the PBSCP expressed CD69, 62% (range 54-67%) of stem cells collected from healthy donors were CD69+, while a median of 19% (range 8-51%) of unstimulated stem cells expressed this marker. Student T-test analysis revealed that unstimulated CD34+ stem cells had a statistically significant (p<0.0001) lower expression of CD69. Conclusions. These data confirm that CD69 is expressed on normal stem cells, that its expression is triggered by stimulation with G-CSF and that CD69 therefore is not an appropriate marker for detection of minimal residual disease in acute leukemia.

In vitro anti-tumoral effect of zoledronate on myeloma plasma cells

PU390
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Bisphosphonates are widely used in multiple myeloma patients mainly for their capacity of inhibiting bone resorption. More recently several authors have showed that bisphosphonates can exert in vitro an antitumor activity towards both plasma and stromal cells, although the exact mechanisms through which they work are not yet defined. In order to assess the antitumoral activity of bisphosphonates, we evaluated the cytotoxic and apoptotic effects of Zoledronic acid on the human myeloma cell line RPMI 8226 and primary plasma cells isolated from bone marrow of patients with multiple myeloma. The purification of myeloma cells was performed by immunomagnetic separation using magnetic beads coated with the mAb BB4. The purity of primary plasma cells was evaluated by standard morphology (May-Grunwald-Giemsa-stained) and by the expression of CD138+ antigen. Only cell population with a purity greater than 95% were used for experiments. RPMI cell line and primary myeloma cells were cultured in RPMI 1640 medium supplemented with 20% FCS and antibiotics; for purified myeloma cells, 2 ng/mL of rIL-6 was added to the culture. A concentration-dependent cytotoxic effect was monitored by use of an MTT assay and radioactive method with 3H-thymidine. The induction of apoptosis was studied in terms of temporal dose response by the identification of morphological features, using DNA-binding dyes Hoechst 33342, and by the detection of cells with plasma membrane alterations (phosphatidylserine translocation) using flow cytometric and microscopic analysis of fluoresceine labelled Annexin V. Treatment for 72 hours with 10, 50, 100 and 500 ?M of Zoledronate induced a progressive inhibition of the proliferation and an increase of cytotoxicity of myeloma plasma cells. Flow cytometry and fluorescence microscopy showed that the observed cell death was mainly due to a time-dependent apoptotic effect. This was more evident in RPMI cell line rather than in freshly obtained myeloma cells from patients in which the programmed cell death was evident after 72 hours of exposure to Zoledronic acid. These results indicate that apoptosis is one of the most important mechanisms through which zoledronate exerts its antitumor effect.

Circulating endothelial cells in systemic sclerosis as a marker of ongoing vascular disease

PU391
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Circulating endothelial cells (CECs) have been described in different conditions of vascular injury. Vascular abnormalities play a key role in the pathogenesis of systemic sclerosis (SSc), that may represent an in vivo model for studying the effects of microvascular damage on CECs. The aim of our study was to quantify CECs and endothelial precursors (CEPs) in SSc patients and to evaluate their clinical and pathogenic role. The study cohort included 40 SSc patients and 40 healthy controls (HC). Five-parameter, 3-color flow cytometry was performed with a FACScan. CECs were
An important issue in allogeneic peripheral blood stem cell transplantation (Allo-PBSCT) is the optimization of the regimen of mobilization of progenitor cells from normal donors. From January 1999 to May 2003, the short-course administration of recombinant human granulocyte colony stimulating factor (r-Hu G-CSF) was evaluated in a total of 60 healthy peripheral blood stem cell (PBSC) donors, 37 male and 23 females, with a median age 46 years (16-63), with a target of collecting >3×10⁶ CD34+ cells per kg of body weight of the recipient (r-Hu G-CSF (lenograstim or filgrastim) was given subcutaneously at a median dose of 9.2 μg/kg per day (range 6.0-15) in two divided doses over 3 days and was followed by analysis of CD34+ cells 12 hours after the last dose. A median of 37.1 circulating CD34+ cells per μL (range, 3.1-185.0) was found in the 60 donors after six doses of r-Hu G-CSF. In 42 donors of our series we performed the first apheresis on the 4th day, achieving a median CD34+ cells collection of 4.6×10⁶ (range 1.3-17.4) per kg of body weight of the recipient (range 1.3-17). A median of 2 procedures (range 0-4) were performed; WBC increased to a maximum of 44×10⁹/L (mean 36.5±10.6) on 4th day and reinfusion of autologous platelet-rich plasma was not necessary in any case. In 68.7% of 42 donors, a single procedure was sufficient to collect the target CD34+ cells and in 16 (50%) we reached the target in the estimated time with a reduced drug exposure, with a lower risk of short-term or long-term adverse effects. The cost of the procedure is widely accepted by healthy donors, as 68.7% of them reach the target in the estimated time with a reduced drug exposure, with a lower risk of short-term or long-term adverse effects. The cost of the procedure is reduced, in terms of both the growth factor administration and the number of apheresis procedures.

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PU393

CLINICAL ISOLATION AND FUNCTIONAL CHARACTERIZATION OF CORD BLOOD CD133+ STEM CELLS

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Background: Human cord blood CD133+ hematopoietic stem cells (HSCs) were isolated using immunomagnetic microbeads coated with the relative monoclonal antibody by means of the CliniMACS → clinical cell isolator. Design and Methods: Closed disposable sets created for clinical purposes were used to process 8 different samples of RBC-depleted cord blood nucleated cells. Different mixtures of human cytokines for hematopoietic and non-hematopoietic stem cells were tested for their ability to generate either nascent hematopoietic stem/progenitor cells or CD45- non-hematopoietic cells. Freshly isolated CD133+ cells were conditioned in culture medium specifically tested to support in vitro myogenesis and osteogenesis. Results: Isolation procedures allowed the recovery of an average 3.16×106 CD133+ stem cells (with a percent mean recovery equal to 109) and a final sample purity of 82%. Purified CD133+ cells had high cloning efficiency, relevant long-term activity and were capable of repopulating irradiated NOD/SCID mice. In 10-day stroma-free cultures, a 2-fold and 8.3-fold expansion of colony-forming cells (CFC) and ELTC-IC, respectively, was obtained; no CD45- cells developed under any culture condition, including hematopoietic and non-hematopoietic cytokines. CD133+ cells underwent commitment towards large nucleated cells expressing either myosin D or osteopontin (as revealed by RT-PCR and immunocytochemistry), with a protein/mRNA expression comparable to or even higher than that observed in cord blood CD133- nucleated cells in identical culture conditions. Conclusion: Collectively, clinical-scale isolation of cord blood CD133+ cells provides a relevant amount of primitive hematopoietic stem cells with high hematopoietic activity and putative mesenchymal in vitro potential.

The hematopoietic stem cell (HSC) compartment encompasses cell subsets with heterogeneous proliferative and developmental potential. Numerous CD34- cell subsets have been described and characterized within human umbilical cord blood (UCB) which might reside at an earlier stage of differentiation than CD34+ HSCs. We identified a novel subpopulation of CD34+CD133-CD7-CD45dimlineage(lin)- HSCs contained within human UCB that were endowed with low but measurable extended long term culture-initiating cell (ELTC-IC) activity. Exposure of CD34+CD133-CD45dim-CD7-lin- HSCs to stem cell factor (SCF) preserved cell viability and was associated with (i) concordant expression of the stem-cell associated Ags CD34 and CD133, (ii) generation of CFU-granulocyte macrophage, BFU-E and Meg-aggregates, (iii) significant ELTC-IC activity, and (iv) upregulation of mRNA signals for myeloperoxidase. At variance with CD34+lin- cells, CD34-CD133+CD45dimCD7-lin- HSCs maintained with IL-15, but not with IL-2 or IL-7, proliferated vigorously and differentiated into a homogeneous population of CD7-CD45+CD25-CD44+ lymphoid progenitors with high expression of the T-cell-associated transcription factor GATA-3. Although they harbored non-clonally rearranged TCRγ genes, IL-15-primed CD34+CD133+CD45dim-CD7-lin- HSCs failed to achieve full maturation, as manifested in their CD3-TCRαβ/γδ- phenotype. Conversely, culture on stromal cells supplemented with IL-15 was associated with the acquisition of phenotypic and functional features of NK cells. Collectively, CD34+CD133-CD7-CD45dim-lin- HSC from human UCB displayed an exquisite sensitivity to IL-15 and differentiated into lymphoid/NK cells. Whether the transplantation of CD34-lin- HSCs possessing T/NK-cell differentiation potential may impact on immunological reconstitution and control of minimal residual disease after HSC transplantation for autoimmune or malignant diseases remains to be determined.

PU395

FUNCTIONAL ANALYSIS OF DENDRITIC CELLS DERIVED FROM PNH DEFECTIVE MONOCYTES IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder of hemopoiesis characterized by the presence of a mutation in the PIg-A gene, responsible for the glycosyl-phosphatidyl-inositol (GPI) anchor synthesis. This defect, affecting clonal hamato-
A LINK WITH HEART FAILURE?

Flow cytometry as CD34+ cells co-expressing AC133 fraction and troponin I. HF was defined as the presence of dyspnea at rest, pulmonary rales, congestion, and even complete and sustained eradication of disease. The analysis of peripheral blood of PNH patients reveals a preferential expansion for the GPI defective granulocyte/monocyte clones. Moreover, a pivotal role in defining the general criteria of the immune response of naïve T lymphocytes against pathogens is played by a monocyte-derived population represented by dendritic cells (DC). The aim of this study was to investigate the ability of GPI defective monocytes, derived from PNH patients, to differentiate in vitro into functional DC. Our data indicate that GPI-monocytes, cultured for a three-five day period in the presence of Interleukin-4 (IL-4) and GM-CSF, fail to express surface DC markers as represented by CD1a. At variance, high levels of CD86 costimulatory molecule, unmodified by LPS treatment are expressed. This phenotype is associated with impaired ability to costimulate TCR triggered T cell proliferation, while a normal phenotype is associated with impaired ability to costimulate T cell proliferation, while a normal LPS as well as CD40L-dependent TNF-α production is observed. These data suggest that PNH-derived DC could mediate defects in antigen-dependent, naïve T-cell activation, contributing to the increased susceptibility to infections observed in PNH patients.

Laboratory and clinical evidence suggests that endothelial progenitor cells (EPC) might have a role in endothelial and tissue. To investigate this in patients with acute myocardial infarction (AMI), we monitored CD34+ cell elevation and correlated the kinetics of CD34+ mobilization with circulating EPCs (identified by flow cytometry as CD34+ cells co-expressing AC133 and VEGFR-2), CFU-End, left ventricular (LV) function, occurrence of heart failure (HF) and B-type natriuretic peptide (BNP) elevation as a marker of LV function. In the whole series of patients, CD34+ cells peaked on day 10, with a mean 48% increase. At peak, CD34+ cells showed a high correlation with EPC as assessed both by flow cytometry ($r=0.7753$, $p<0.001$) and CFU-End ($r=0.6837$, $p=0.001$), but no correlation with age; C-reactive protein and clinical parameters of infarct extension (CK-MB and troponin I, LV ejection fraction or wall motion score index). Eleven patients satisfied the criteria for HF. Absolute CD34+ cell counts on days 10 (4.14±1.7 vs. 6.3±2.4, $p<0.03$) and 14 (3±1.1 vs. 4.5±1.5, $p<0.05$), percentage of CD34+ cell increase (37%±13% vs. 51%±12%, $p<0.001$) were significantly reduced while BNP levels (314±436 vs. 111±84 pg/mL, $p<0.002$) were more elevated in patients with HF than those without HF. Peripheral CD34+ cell counts were similar at entry for patients with or without HF. Furthermore, BNP showed a strict negative correlation with percentage of CD34+ increase ($r=-0.73$, $p<0.001$). Our data suggest that patients with HF during hospitalization have a significantly reduced mobilization of CD34+ cells compared to uncomplicated patients. Given the strict correlation, in the peripheral blood, between CD34+ cells and EPC, our results emphasize the need for observational longitudinal clinical trials assessing the prognostic role of CD34+ cells/EPC mobilisation during AMI and reinforce the interest for the ongoing clinical trials investigating the use of angiogenic growth factors aimed at increasing EPC mobilisation during AMI.

Factors influencing the collection of peripheral blood stem cells (PBSC) in patients with hematologic malignancies: A single center experience

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Summary: Peripheral blood stem cells (PBSC) are increasingly used in association with high-dose myeloablative therapy to achieve more durable remissions and even complete and sustained eradication of disease in patients with hematologic malignancies. However, this procedure, due to several factors including previous chemotherapy regimens, is often limited by an inadequate collection of PBSC. The purpose of the study was to identify factors influencing mobilization and PBSC collection in an effort to optimize the harvesting procedure. Patients and methods: Seventy-seven con-
Electronic patients (33 females, 44 males) with hematologic malignancies (7 AML, 9 ALL, 1 CLL, 13 HD, 25 NHL, 18 MM) were analyzed. Median age was 47 years (range 13-69). Thirty-two pts were in CR, 27 in PR and 18 in advanced stage (1 non-responder, 1 disease progression and 15 relapse) following first or second-line treatment (8 pts received more than 3 lines of therapy). Twenty-three pts had bone marrow involvement at the time of mobilization. Two patients underwent further mobilization. Several regimens were used: DHAP (=27), intermediate-dose Cyclophosphamide (CY=19), MACOP-B (=3), CHOP (=1), ABVD (=7), consolidation regimens as MINI-ICE, DIA, HAM (=16), all followed by administration of G-CSF (5-10 microg/kg/day) until the completion of PBSC collection. G-CSF alone was given to 4 pts. Erythropoietin (EPO) was administered in association with G-CSF in 7 pts. A median number of 2 leukaphereses per patient (range 1-3) was performed on median day +10 (range 4-19) and +11.5 (range 7-20) from the end of treatment, respectively, when CD34+ cell count was >10/mL. The median CD34+ count was 53.3/ml (range 15.1-112) in pts submitted to one apheresis versus 16.4/mL (range 13.5-17.5) in patients submitted to 2 aphereses. The median number of CD34+ cells/10^6/kg collected was 3.6 (range 0.13-54) after a single apheresis versus 2.1 CD34+ cells/kg (range 0.4-12.5) after two and 0.37 CD34+ cells/kg after three aphereses. Median peripheral white blood cell counts (WBCx10^9/L) pre-harvest was 9250 (range 1000-71400) for the first and 16400 (range 2940-376000) for the second PBSC collection. Median CD34+ cell counts/mL pre-apheresis were 38.6 (range 14.5-499.4) for one vs 28.1 (range 11.7-162.7) for two aphereses, respectively. Results. The adequacy of the PBSC harvest was not associated with age, gender, past or present bone marrow involvement or disease status, number of cycles of previous therapy, prior radiotherapy, administration of EPO. In contrast, acute leukemia patients mobilized in remission had a greater yield of CD34+ cells compared with myeloma patients (p=0.0009). Significant difference between mobilizing regimens was reported for DHAP versus CY (p=0.0118) or other protocols (p=0.0348) with a greater amount of CD34+ cells collected. The white cell count on the day of harvest and the absolute number of circulating CD34+ cells strongly correlated with the CD34+ cell of harvest and the absolute number of circulating CD34+ cells. However, chemotherapy itself may be predictive of a higher CD34+ yield when administered DHAP regimen. A tendency towards a greater collection efficiency was observed in acute leukemia patients. The number of CD34+ cells harvested in a single leukapheresis can be predicted by measurement of the peripheral-blood circulating CD34+ concentration on the day of harvest. This correlation may facilitate an efficient organization of leukapheresis procedures.

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PU398
CHARACTERIZATION OF A TRILINEAGE (MEGAKARYOCYTIC-ERYTHROID-MAST) PROGENITOR CELL LINE OBTAINED FROM GATA-1 LOW MICE
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GATA-1 low mice, obtained through the targeted deletion of upstream regulatory sequences of the GATA-1 locus that causes the selective abrogation of GATA-1 expression in megakaryocytes (Mk), present only a moderate impairment of erythroid (E) differentiation due to an increased rate of E progenitor apoptosis (Vannucchi AM et al, Blood 2001;97:3040), while they experience a severe life-long thrombocytopenia in spite of massive MK accumulation in hematopoietic tissues because of the defective platelet release from GATA-1 deficient Mk. Furthermore, they develop with age a myelofibrosis-like syndrome characterized by many of the phenotypic features of the human disease (Vannucchi AM et al. Blood 2002; 100:1123). We have also provided substantial evidence that mast cell (MC) lineage differentiation is impaired in GATA-1 low mice, that present increased number of (i) morphologically abnormal alcinian bluepos MC and (ii) apoptotic metachromatic MK precursors in the connettive tissues and in peritoneal lavage (Migliaccio AR et al, J Exp Med 2003; 197:281-296). The bone marrow and the spleen of GATA-1 low mutants contain a unique trilineage progenitor, the CFU-E/MK, that gives rise in 7 days to large abnormal colonies containing not only erythroblasts (benzidinepos, TER-119pos) and megakaryocytes (acetylcholinesterasepos, 4A5pos), but also mast cell precursors (methachromaticneg granul, c-kithigh/CD34high). We now report on the successful isolation of continuous factor-dependent cell lines that were derived with an efficiency of >90% by culturing in limiting dilution assay the abnormal trilineage colonies derived from the spleen of GATA-1 low mice. One of these cell lines, SN1, has been repeatedly cloned and maintained in culture up to one year, and has been further characterized. SN1 cells are IL3/SCF-dependent for their growth while both EPO and TPO alone were unable to sustain growth; CD34/Kit are coexpressed in >80% of cells, with a minority of them being also Ter-
119pos and 4A5/2D5pos. Most cells were Alcian blue-pos while about 10% were AchEpos and routinely benzidineneg. Extensive RT-PCR analysis of gene expression confirmed that these cells did express most of lineage-associated genes of E, M k, and MC lineage, while the myeloid-restricted myeloperoxidase gene was not expressed. Overall, these observations indicate that the hematologic phenotype of GATA-1low mice is characterized by the contemporary involvement of E, M k, and MC lineages, and suggest that the cell targeted by the abnormal GATA-1 expression may be a trilineage progenitor that is clonally expanded in the SN1 cell line; the latter, may represent an unique tool for dissecting GATA-1 dependent molecular events governing the fate decision towards these three hematopoietic lineages.

**PU399**

**IMPACT ON NEWBORN HEMOPOIESYS OF TWO THERAPY PROTOCOLS ADMINISTERED DURING PREGNANCY TO REDUCE THE ABORTIVE RISK IN MOTHERS WITH AUTOIMMUNE DISEASE**

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The aim of the study was to evaluate hematologic data, RBC, WBC, platelets and peripheral progenitor hematopoietic colony numbers, in new-borns from mothers affected with severe autoimmune diseases and treated in pregnancy with two different therapies to avoid the abortive risk: acetylsalicylic acid 100 mg/day (A) or ASA 100 mg/day (B). The peripheral blood was collected at birth from 11 evaluable subjects (5 males and 6 females) only (B). The proliferative activity was expressed as percentage of standard growth, considered as 100%.

Table 1. Mean±Standard Error (SE) of RBC, WBC, Platelets (Plt) and hematopoietic progenitors in 11 evaluable samples.

<table>
<thead>
<tr>
<th></th>
<th>RBC</th>
<th>WBC</th>
<th>Pt.</th>
<th>BFU-E</th>
<th>CFU-GM</th>
<th>CFU-GEMM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: 6 cases</td>
<td>100</td>
<td>155±106</td>
<td>158±114</td>
<td>117±71</td>
<td>163±116</td>
<td></td>
</tr>
<tr>
<td>B: 5 cases</td>
<td>100</td>
<td>160±68.3</td>
<td>121±38</td>
<td>110±35</td>
<td>160±60</td>
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</table>

The proliferative activity was evaluated after incubation with hydrocortisone (10, 100 e 1000 ng/mL), cyclosporin A (10 ng/mL) and GM-CSF (10 ng/mL). In all these conditions the BFU-E and CFU-GM number was always lower than in standard controls in group A, on the contrary their number was higher than basal test in group B (Table 2).

Table 2. BFU-E and CFU-GM growth analyzed after in vitro exposure to hydrocortisone (Hy), cyclosporin A (CY-A) and GM-CSF. The proliferative activity is expressed as percentage of standard growth, considered as 100%.

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Hy 10 ng/mL</th>
<th>Hy 100 ng/mL</th>
<th>Hy 1000 ng/mL</th>
<th>Cy A 10 ng/mL</th>
<th>GM-CSF 10 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFU-E A 5 cases</td>
<td>157±23</td>
<td>97±28</td>
<td>66±24</td>
<td>59±14</td>
<td>75±21</td>
<td></td>
</tr>
<tr>
<td>BFU-E B 5 cases</td>
<td>150±116</td>
<td>160±60</td>
<td>121±38</td>
<td>110±35</td>
<td>160±60</td>
<td></td>
</tr>
<tr>
<td>CFU-GM A 5 cases</td>
<td>137±30</td>
<td>131±21</td>
<td>150±114</td>
<td>117±71</td>
<td>163±116</td>
<td></td>
</tr>
<tr>
<td>CFU-GM B 5 cases</td>
<td>150±143</td>
<td>137±30</td>
<td>131±21</td>
<td>150±114</td>
<td>117±71</td>
<td>163±116</td>
</tr>
</tbody>
</table>

Our data indicate that therapy administered during pregnancy is able to modify the hematopoietic behaviour in newborns. With ASA +flucortolone therapy (A) a lower RBC count coupled with higher standard BFU-E numbers were observed. These progenitors showed also a low response to all the in vitro treatments utilized. WBCs were quite the same in A as in B group. In B the CFU-GM maintained a correct reply to in vitro stimulators (Hy 10 and 100 ng/mL, GM-CSF) or to potentially suppressive substances (Hy 1000 ng/mL, Cy A). After ASA treatment higher RBC numbers and lower BFU-E counts were detected in standard conditions. These progenitors and the respective CFU-GM counterparts showed always the capability to increase their proliferative activity. This behaviour could be suggestive of immunosuppressive influence of maternal origin that is corrected by Hydrocortisone and Cy A. These immunosuppressive effects seem to be absent with association of ASA+flucortolone. These data may have an impact on therapeutic decisions during pregnancy to control high abortive risk due to mother's autoimmune disease.

**PU400**

**LENOGRASTIM AND FILGRASTIM EFFECTS ON NEUTROPHIL POLARIZATION AFTER MOBILIZATION IN PATIENTS UNDERGOING A-PSCT: EVALUATION BY COMPUTER ASSISTED IMAGE ANALYSIS**

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An abnormal microtubular assembly, which correlates which the inability of cells to achieve a fully bipolar shape under stimulus, is the first cause of a defective neutrophil chemotaxis. In the same way, a basal hyperactivation of cytoskeleton may result in a hyper-polarization with loss of deformability and defective response to chemotactic stimulus. It is known that rh-GCSF is able to interfere with neutrophil chemotaxis, and different kinds of rh-GCSF act in different ways (Azzarà A., Am J Hematol, 2001). As regards polarization, few authors evaluated this function, which was evaluated by ordinary light microscope. This subjective technique has been recently assessed to be the most sensitive and reliable polarization assay (Kalkman, P.M.J., Clin Exp Immunol, 2002). So we studied the effects of Chinese Hamster ovary-derived glycosylated rhG-CSF (Lenograstim) and E.Coli-derived non-glycosylated rhG-CSF (Filgrastim) on circulating neutrophils from patients after rh-GCSF mobilization in order to perform autologous peripheral stem cell transplantation. 8 patients (4 with Multiple Myeloma, 3 with non-Hodgkin’s Lymphoma, 1 with Chronic Lymphocytic Leukemia) were treated by Filgrastim; 8 patients (5 with Multiple Myeloma, 2 with non-Hodgkin’s Lymphoma, 1 with chronic lymphocytic leukemia) were treated by Lenograstim. The schedule was: 5 \( \mu \text{g/kg/day} \) subcutaneously from day 1 to day 6, than 10 \( \mu \text{g/kg/day} \) on day 7, 8, ..., until mobilization of the requested CD34+ amount. On day 8, neutrophils were collected, separated on density gradient and placed in Boyden chambers on micropore filters. A brief (10 minutes) priming was carried out putting in the lower compartment F-mpl 10^{-8} (or buffered saline as control), in order to evaluate the first events which precede the start of chemotaxis. After incubation, the filter were removed, fixed, dehydrated, stained, diaphanized and analyzed by an image analysis work-station. The device employs a TV-camera mounted on a Leitz microscope, a PC equipped by a MGA Matrox Millennium Video Board (Matrox Electronic Systems) especially adapted. In particular, the Blob Analysis was used. The software, after an analogical-digital transformation of the images containing the neutrophils under study, is able to perform in real time: classification, segmentation, setting of foreground touching, minimal and maximal area, x and y centroid, breadth, compactness and, in particular for this study, elongation. This parameter is identified by minimum (1 = perfectly round cell), maximum, mean, std deviation, variance, min to pass, max to pass (according to threshold and filters), total count. Results. Filgrastim induced neutrophils in basal condition displayed Elongation mean value of 2.859±0.891; Lenograstim induced neutrophils in basal condition displayed Elongation Mean Value of 2.604±0.604: \( p = 0.02 \). Filgrastim induced neutrophils under stimulus displayed elongation mean value of 2.899±2.537, without any enhancement if compared with basal conditions: 453 vs 428 images totally evaluated (p=n.s.) On the contrary, Lenograstim induced neutrophils under stimulus displayed Elongation Mean Value of 2.800±1.907: 615 vs 609 images totally evaluated (p=0.01 if compared with basal conditions). These data shows that Filgrastim induced neutrophils are already activated and seems to be no more responsive to further chemotactic stimuli. On the contrary Lenograstim induced neutrophils showed to be less elongated in basal condition and more responsive to the applied stimulus, acting in a more physiological way. It is possible that the glycosylation of Lenograstim, which makes it more similar to the natural one, play a role in this different activity. This difference should be taken in account, in order to preserve as better as possible all the functions of the induced neutrophils.

PU401
HER-1 TRANSACTIVATION THROUGH MONOCYTE AND CANCER CELL 7-TMD RECEPTORS
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The prototypic ligand for HER-1 tyrosine kinase is heparin-binding EGF-like growth factor (HB-EGF), an EGF superfamily member, which is produced by monocytes and normal or neoplastic epithelial cells. The HER-1/HB-EGF interaction following the proteolytic release of the growth factor supports mitogenic activities and regulates differentiation pathways related to muscles and neurons with pathogenetic relevance in a number of conditions. Monocytes express the 7-TMD receptor CXCR4 on their membrane and produce HB-EGF. We argued that monocytes, epithelial or neoplastic cells recruited locally through CXCR4-mediated chemotactic activity may be also induced to release HB-EGF resulting in mitogenic activity and differentiation signals. First, we studied this in monocytes using RT-PCR, Northern blot, flow cytometry, ELISA and functional transwell or cell-to-cell approaches. CXCR4 stimulation of monocytes with its ligand CXCL12 induces metalloproteinase activation, cleavage and release of HB-EGF (as shown also by flow cytometry) and rapid (10 to 20 minutes) transactivation of cells expressing HER-1, as evaluated by phosphorylation of tyrosine 1068 on HER-1 cytoplasmic tail in HeLa cells. This was observed in transwell stimulation, whereas cell-to-cell contact did not lead to similar transactivation. Following this rapid phenomenon, stimulation with CXCL12 led to increased amounts of mRNA for HB-EGF up to 24 hours. The same events can be reproduced using CXCL12-conditioned, monocyte-derived culture supernatant, further showing that monocytes release HB-EGF after stimulation with CXCL12. This provides evidence that monocytes may influence the activity of bystander HER-1-positive cells.
in terms of proliferation and/or differentiation. Experiments on epithelial neoplastic cells (for instance, the human bladder cancer cell line 5637) gave similar results. Thus, one and the same regulatory motif involving chemokines, their 7-TMD receptors, growth factors such as HB-EGF and their tyrosine kinase receptors may dominate regeneration and healing, proliferation in degenerative lesions as well as carcinoma or metastasis growth and represents a candidate target for therapy in these conditions.

PU402
EXPRESSION OF THE 67 KDA LAMININ RECEPTOR ON PROGENITOR CELLS AFTER MOBILIZATION WITH GRANULOCYTE-COLONY STIMULATING FACTOR IS INVOLVED IN THEIR ADHESION TO STROMAL MICROENVIRONMENT

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Allogeneic hematopoietic stem cell transplantation is increasingly performed with blood-derived cells because of technical advantages and shorter time to engraftment. The homing of intravenously infused stem cells into the bone marrow is a complex process that primarily depends on the modulation of cell-surface expression of adhesion molecules and their corresponding ligands on stem and stromal cells. Cancer cells attach to basement membranes via specific cell surface laminin receptors and may degrade components of the extracellular matrix by secreting proteases. The 67-kDa laminin receptor (67LR) is a nonintegrin protein with high affinity for laminin, which plays a critical role in basement membrane invasion and metastasis of cancer cells. Expression of the 67LR is increased in a variety of human carcinomas and directly correlates with enhanced invasive and metastatic potential. In analogy with the migration network could be the reason of the defect of proliferation and differentiation typical of myelodysplastic syndromes (MDS). Patients and Methods: We evaluated, at diagnosis, the hematopoietic growth factors serum levels of 120 patients affected by MDS (M/F 74/46; median age 65; FAB: 50 AR, 20 ARSA, 25 ABER, 12 ABER-T, 13 LM MC, 55.7 g/dL, WBC 5.1 × 10^9/L, PLT 124 × 10^11/L, p < 0.001). In conclusion, our results indicate that 67LR expression on human CD34+ cells allows them to adhere to laminin. In addition, 67LR up-regulation on mobilized human CD34+ progenitor cells and the inhibition of LTC-ICs after 67LR competition also suggest that 67LR is able to promote lodgement and proliferation of bone marrow progenitor cells within the BM microenvironment.

PU403
SERUM LEVELS OF HEMATOPOIETIC GROWTH FACTORS IN MYELODYSPLASTIC SYNDROMES
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Background: Anomalies of hematopoietic cytokines network could be the reason of the defect of proliferation and differentiation typical of myelodysplastic syndromes (MDS). Patients and Methods: We evaluated, at diagnosis, the hematopoietic growth factors serum levels of 120 patients affected by MDS (M/F 74/46; median age 65; FAB: 50 AR, 20 ARSA, 25 ABER, 12 ABER-T, 13 LM MC, 55.7 g/dL, WBC 5.1 × 10^9/L, PLT 124 × 10^11/L, p < 0.001). In conclusion, our results indicate that 67LR expression on human CD34+ cells allows them to adhere to laminin. In addition, 67LR up-regulation on mobilized human CD34+ progenitor cells and the inhibition of LTC-ICs after 67LR competition also suggest that 67LR is able to promote lodgement and proliferation of bone marrow progenitor cells within the BM microenvironment.
and TPO (555±108 vs 126±31 pg/mL, p<0.02) resulted higher compared with those of 30 normal controls. SCF values resulted significantly reduced (795±215 vs 1510±320 pg/mL, p<0.02). In high risk MDS patients we observed SCF, IL-1β, GM-CSF, G-CSF, TPO and EPO levels closer to normal values and particularly elevated IL-8 levels. Low risk MDS were distinguished by higher levels of M-CSF, G-CSF, GM-CSF, EPO, IL-6 (ARSA in particular) and IL-6, while LMM C was characterized by high levels of G-CSF and low levels of EPO. An inverse correlation between Hb and EPO, between PLT and TPO and a linear correlation between TNF and WBC were pointed out. Conclusions. In MDS, serum levels of active cytokines during the late phases of hematopoiesis though appearing high, hardly explain the level of cytopenia; the production of early-acting cytokines seems to be considerably compromised. The high levels of TNF and IL-1 found in most patients could play a role in actively inhibiting hematopoiesis. The blastic transformation leads to further alterations of cytokines’ production. The combination of impaired production of hematopoiesis-activating cytokines and the excessive production of inhibiting cytokines could contribute to the development of MDS.

PU404
Spleen Enlargement Following Recombinant Human Granulocyte Colony-Stimulating Factor Administration for Peripheral Blood Stem Cell Mobilization

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Recombinant human granulocyte colony-stimulating factor (rh-G-CSF) is widely used to mobilize peripheral blood stem cells (PBSC) for autologous or allogeneic transplants, and available data regarding its short- and long-term toxicity have shown no serious adverse effect. However, there have been reports of spontaneous spleen rupture in rh-G-CSF- or cyclophosphamide plus rh-G-CSF-mobilized individuals. We tested the accuracy of ultrasound (US)-calculated (area-length method) spleen volume compared with palpation and US-measured longitudinal diameter in detecting changes in spleen size in two groups of subjects whose PBSC were mobilized by rh-G-CSF- or cyclophosphamide plus rh-G-CSF-mobilized individuals. We tested the accuracy of ultrasound (US)-calculated (area-length method) spleen volume compared with palpation and US-measured longitudinal diameter in detecting changes in spleen size in two groups of subjects whose PBSC were mobilized by rh-G-CSF- including regimens (healthy donors for autologous transplant, and patients with a hematologic malignancy scheduled for autologous transplant). We also correlated the size changes with circulating leukocyte and CD34+ cell counts evaluated on the last day of rh-G-CSF administration; we assessed interobserver variability of US measurements and examined the correlation between US-calculated and computed tomography (CT)-measured spleen volume in a subgroup of patients. Thirteen healthy donors (8 women and 5 men; median age 38 years, range 28-55) received a mobilization regimen of s.c. rh-G-CSF (Lenograstim) 263 micrograms twice a day (median treatment: 6 days), while 22 patients (15 women and 7 men; median age 51.5 years, range 18-63; multiple myeloma (n=11), aggressive non-Hodgkin’s-lymphoma (n=4), acute myeloid leukemia (n=4), Hodgkin’s lymphoma (n=3)) received i.v. cyclophosphamide 7 g/m² (median dose: 10 gr) plus s.c. rh-G-CSF 263 micrograms once a day (median treatment: 12 days). In all subjects, spleen size were assessed on 3 occasions (pre-, during-, and post-rh-G-CSF course). Interobserver variability of US-calculated spleen volume was very low (Pearson value= 0.91); the correlation between the volume calculated by US and that measured by 3-dimensional computed tomography was excellent (Figure 1A). During mobilization, spleen enlargement was detected by palpation in 17% of subjects, by US-measured longitudinal diameter in 60%, and by US-calculated volume in 91%. Median increase in spleen volume was 300 mL (range, 54-820; p<0.001) in healthy donors and 135 mL (range, 0-413; p=0.004) in the patient group; leukocyte and neutrophil counts were significantly higher in healthy donors than in patients. Spleen enlargement correlated with white blood cell count elevation (p=0.016) but not with circulating CD34+ cells (Figure 1B and 1C). One month after the last administration of rh-G-CSF, the median decrease was 160 mL (range, 35-800) in healthy donors and 58 mL (range, 0-310) in patients (Figure 2). No subject reported any discomfort or pain in the splenic area during or after mobilization. Thus, when evaluated by sensitive methods, rh-G-CSF caused spleen enlargement in almost all individuals treated; intrasplenic accumulation of circulating granulocytes and myeloid precursors was the most likely mechanisms by which splenic tissue enlarges. US-calculated volume proved to be an excellent method, much better than longitudinal diameter, for detecting non-palpable splenomegaly induced by rh-G-CSF; it may help to identify donors with greater spleen enlargement (at risk of spontaneous rupture) thus needing close monitoring.

References
Cell Therapy

PU405
GENERATION OF LEUKEMIA SPECIFIC T CELLS FROM HLA-MATCHED SIBLINGS OF LEUKEMIA PATIENTS USING DONOR DENDRITIC CELLS/TUMOR HYBRIDS
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Introduction. Graft-vs-leukemia (GVL) effect following allogeneic blood stem cell transplantation can cure patients with chemotherapy refractory hematologic malignancies. Methods to expand leukemia-specific T cells from healthy stem cell donors for post-transplant infusion could potentially enhance this GVL effect. We evaluated the ability to generate leukemia specific T cell clones in vitro from healthy HLA matched sibling donors by stimulating donor lymphocytes with donor dendritic cells (DCs) either chemically or electrically fused to patient leukemia cells. Methods. Leukemia cells were isolated by leukapheresis from the peripheral blood of four patients (1 ALL, 2 AML blast crisis, and 1 CML- chronic phase). Immature DCs were generated from HLA matched siblings of leukemia patient by culturing donor monocytes for 7 days in 10% human AB serum with GM-CSF (1000 IU/mL) and IL-4 (1000 IU/mL). DC/tumor hybrids (DCTH) were generated by fusing donor DCs to patient leukemia cells by two different techniques: 1) 1 ml of polyethylene glycol (PEG/DMSO solution) was added to a cell pellet containing DCs and tumor cells in a 1:1 ratio; 2) Electro-pulsing DCs and tumor cells in a 1:1 ratio was accomplished by first aligning at 40 V for 5 seconds and then a pulse of 1000 V/cm at 25 mF. T cells obtained from the HLA-identical siblings of leukemia patients were stimulated in vitro weekly x 2 by one of three different stimulator populations: 1) irradiated patient tumor cells alone 2) electro-fused DCTH 3) PEG fused DCTH. Donor T cells were then cloned from bulk cultures by limiting dilution and more than one hundred clones from three separate leukemia patients were tested for their ability to recognize patient leukemia cells by an ELISA measuring interferon \( \gamma \) (IFN-\( \gamma \)) secretion or cytotoxicity. Results. DCs and leukemia cells were stained with red and green fluorescent cell membrane dyes and were analyzed by FACS following PEG or electropulsion. Both techniques resulted in a reproducible fusion efficiency of 5-15%. Donor T cells stimulated by patient leukemia cells typically failed to expand. In contrast, donor T cells stimulated by DCTH proliferated rapidly following each stimulation. In 3 of the 4 donors studied, T cell clones which secreted IFN-\( \gamma \) when co-cultured with patient leukemia cells were identified. While the majority of these clones recognized both leukemia cells and nor-
mal patient hematopoietic cells, T cell clones with leukemia specificity (determined by secretion of IFN-γ or cytotoxicity by Chromium51 release) were isolated and expanded from sibling donors. Conclusions. Leukemia specific T cells can be generated in vitro from healthy HLA matched siblings using donor DCs which are either chemically or electrically fused to patient tumor cells. DCTH could be used for the in vitro expansion of donor T-cells (both CD4+ and CD8+) with leukemia specificity to be infused post-transplant for future leukemia-targeted allogeneic transplant trials. Moreover tumor-specific clones generated employing DCTH can potentially be used for the identification of leukemia-specific antigens for tumor rejection.

PU406
INTERLEUKIN-2 INCREASES THE SUSCEPTIBILITY OF FRESHLY ISOLATED NEOPLASTIC B CELLS TO RITUXIMAB-MEDIATED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY IN VITRO
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Current evidence suggests that the chimeric anti-CD20 antibody rituximab works in vivo mainly through complement dependent cytotoxicity (CDC) and/or antibody dependent cellular cytotoxicity (ADCC). We characterised the ADCC activity of rituximab in vitro against freshly isolated neoplastic B cells and using different effector cell populations. Effector cells included peripheral blood mononuclear cells (PBMC), purified CD56+/CD3− cells, or PBMC cultured for 48 hours in medium containing 10% FCS and 1000 U/mL rhIL-2. Targets included 5 fresh cases of chronic lymphocytic leukemia (B-CLL), 2 mantle cell lymphoma (MCL), 1 prolymphocytic leukemia (PLL) and 1 follicular lymphoma (FL). Furthermore two lymphoma cell lines (BJAB and MEC2) were used as controls. None of the primary leukemic samples examined was lysed significantly by freshly isolated PBMC in absence or presence of rituximab, even at 63:1 effector:target ratios. On the contrary, PBMC, which contained a mean of 10% NK cells, could lyse the B lymphoma cell lines (BJAB, MEC2) to about 40% in the same experimental conditions. Peripheral blood CD56+/CD3− NK cells purified to 95% could lyse B lymphoma cell lines to 70% and primary leukemic samples to 25-30%, at E:T ratios of 63:1. Interestingly, PBMC cultured for 48 hours in presence of rhIL2 became very strong effector cells in ADCC assays using primary leukemic cells as targets: IL-2–activated PBMC together with rituximab induced lysis of all leukemic samples by 15-50% above the background in the absence of antibody (mean 30%). Culturing PBMC in IL-2 did not increase the percentage of NK cells present relative to the percentage in the freshly isolated cell population. We conclude that primary leukemic cells are more resistant than leukemic cell lines to rituximab mediated ADCC in vitro using peripheral blood derived NK cells, regardless of CD20 expression levels. Culturing PBMC in IL-2 allows NK cell activation, but not expansion, leading to greatly increased ADCC using freshly isolated leukemic targets. These data suggest caution is needed when interpreting data obtained with cell lines rather than freshly isolated leukemic samples. Furthermore, the marked effect of IL-2 for only 48 hours suggests that IL-2 stimulation in vivo or ex vivo may significantly increase the therapeutic activity of rituximab.

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PU407
IN VITRO AND IN VIVO MYOGENIC CONVERSION OF HUMAN BONE MARROW CELLS
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Bone marrow mesenchymal stem cells (MSCs) have been shown to generate myotubes or cardiomyocytes in vitro using 5-azacytidine or amphotericin B (Wakitani, 1995; Phinney, 1999; Makino, 1999). This phenomenon, probably linked to the activation of phenotype-specific genes, is nevertheless sporadic and the efficiency of myogenic conversion is usually low. We generated muscle cells from unfractioned human bone marrow (BM) cells obtained from ribs resected at the time of thoracotomy in 36 lung cancer patients and from 29 iliac crest BM of healthy donors for allogeneic transplantation. Cells extensively flushed out from the rib and low-density mononuclear cells from BM aspirates from the iliac crest were grown in DMEM 10% FCS without any chemical induction and the supernatant of the cultures was harvested weekly and replated in new flasks without medium changes. Flow cytometry, PCR and western blot analyses performed on both adherent and non-adherent fractions at different time points showed a variable expression of myogenic markers. Starting from the third adherent fraction, numerous long multinucleated cells (myotubes) were observed in 7 out of 36 (19.4%) bone marrow samples taken from ribs and 1 out of 29 (3.4%) bone marrow samples taken from the iliac crest. Myotubes expressed...
the majority of myogenic markers by Immunocytochemistry. Injection of whole BM into NOD-RAG xenograft-permissive immunodeficient mice resulted in the engraftment of human cells (as demonstrated by immunohistochemistry and FISH) and in their differentiation into mature myofibers expressing human dystrophin. Further experiments are ongoing to characterize these myogenic precursors and to explore their transplantability into immunodeficient murine hosts.

PU408
FEASIBILITY AND SAFETY OF EXTRACORPOREAL PHOTOCHEMOTHERAPY IN SMALL CHILDREN WITH RESISTANT GRAFT-VERSUS-HOST DISEASE
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Extracorporeal photochemotherapy (ECP) is a therapeutic approach based on the biological effects of ultraviolet light-A (UVA) and psoralens on mononuclear cells collected by apheresis. Indication for ECP include treatment of oncological and autoimmune diseases; furthermore ECP has been shown to be an effective therapy for patients with acute and chronic graft-versus-host disease (GVHD) following allogeneic bone marrow transplantation and in the management of rejection of heart and/or lung transplants. This therapy involves separating the leukocyte-rich fraction from whole blood and treating with psoralen and UVA exposure with the UVAR XTS automated system (Therakos) for both the collection and the photoradiation. This procedure is less easily performed in pediatric and, above all, in small pts (weighing less than 20 kg) due to several technical difficulties including vascular access and excessive extracorporeal volume. The aim of our study was to evaluate a technique with two independent steps which permits this therapeutic approach in all low weight pts. Over the past two years we have performed 210 ECP in 14 children (median age 6 yrs, median weight 15 kg) with rejection of heart and lung transplants and acute or chronic GVHD. In small pts the procedure consists first in collection of mononuclear cells by a Cobe Spectra continuous blood cell separator and, secondly, irradiation in an independent machine PUVA Combi Light Irradiator. The central venous access in pts. < 20 was via a double-lumen catheter. For these pts the separator was primed with leukocyte-poor and irradiated red blood cells and two blood volumes were processed. The blood flow rate was between 15 and 30 mL/min and ACD-A anticoagulant rate from 1:13 to 1:14. The mean mononuclear cells collection was 1,8x10^6, Hct from 4 to 5%. The final harvest volume (50-80 mL) added with saline was then treated with 8-MOP (200 ng/mL), irradiated with UVA light (2J/cm^2) and reinfused within 1 h into the pts. No severe side effects were documented during the procedures. In conclusion, ECP can be safely and easily used in small patients when performed by an experienced apheresis team.

PU409
PERIPHERAL LYMPHOCYTE SUBPOPULATIONS AFTER LONG-TERM EXTRACORPOREAL PHOTOCHEMOTHERAPY IN PATIENTS IN REJECTION AFTER LIVER TRANSPLANTATION
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Extracorporeal photochemotherapy (EPC) is an immunomodulatory technique in which lymphocytes are reinfused after exposure to a photoactive compound and to a ultraviolet light. ECP showed efficacy in the treatment of cutaneous T-cell lymphomas, GVHD, autoimmune diseases, and allograft rejection. We performed a preliminary study to assess the safety and the efficacy of EPC in the acute and chronic rejection in liver transplantation; particularly we studied the lymphocyte subpopulation in order to evaluate the immunomodulatory effects and the risk of immunotoxicity. We selected 10 recipients of liver transplant in rejection phase or in high risk of rejection, not responsive to conventional immunosuppressive therapy. They received ECP treatment, with UVAR XTS system (Terakos), for two consecutive day initially every week, then every two weeks and finally every month as maintenance. The lymphocyte subpopulations CD3+, CD4+, CD8+, CD19+, CD16/56+CD3+ (NK) were evaluated before to start the therapy and after 4 or 6 months of treatment; we used a four colour flow cytometric technique (FacsCalibur, BD Biosciences). Among our patients, 8 reach the sixth month of therapy and showed a clinical improvement with a reduction of the liver enzymes and of the viral load (in HCV+ subjects). Two patient stopped the EPC after three months for absence of clinical results. The blood concentration of lymphocyte CD3+, CD4+, CD8+, CD19+ do not showed any relevant modification, while we evidenced a an increase of NK cells (from 66±105/mL to 94±131/mL, p=0.01); 7 patients presented an increase of NK and 3 subject had a stable number of this population. The ratio CD4/CD8 don't showed any substantial modification, even if there is a trend to the reduction in accordance to other investigations. These results suggest that EPC doesn't induce significant T cell depletion and stimulates the NK population; this aspect is interesting
because some authors assert that a depressed NK activity may cause a low therapeutic response. Moreover, these data highlight the prevalent immunomodulatory effects of ECP. Even if the immunological mechanism of action of EPC remains undefined and, probably, multiple factors interact to produce the therapeutic effects, the fact that this therapy has minimal adverse effects and is without evidence of lymphotoxicity, indicates that it may be an important choice to chemotherapeutic and immunosuppressive agents.

PU410
PHASE I CLINICAL TRIAL WITH CYTOKINE INDUCED KILLER CELLS FOR PATIENTS WITH ADVANCED LYMPHOMA: PRELIMINARY DATA
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Immunotherapy represents a promising treatment strategy for many types of cancer. The goal of this kind of therapy is to stimulate immune system to recognize and kill cancer cells by modifying the host response. CIK cells are highly efficient cytotoxic effector cells capable of lysing a broad range of tumor cell lines. We report our experience in large-scale ex vivo expansion of CIK cells and present preliminary data from the first 3 patients treated in our ongoing phase I safety trial. 9 PBMcs samples were obtained from 3 patients with NHL in progressive disease. CIK cells were generated under GMP guidelines by growing PBLs in the presence of IFN-γ followed by IL-1β, OKT3 and IL-2 and incubated in LifeCell -> culture bags at 37°C. Expansion was assessed between day 21 and 25. Flow cytometric analysis was performed weekly. The cytotoxic activity of CIK cells was investigated in LDH release assay, against B-cell lymphoma target cell lines. All cultures were analysed for the presence of abnormal transcripts by RT-PCR from RNA extracted from PBMcs. Results: The starting cell population contained a median of 52.3% CD3 and 3.2% CD3+CD56+ cells. After 21 days of expansion, the median culture was 98% viable, the median content of CD3+ cells was 95.2%, for CD3+CD56+ cells was 33% (22%-64.3%). This represents a median expansion of CD3+ cells of 161.8-fold and of CD3+CD56+ 494-fold. At a 20:1 and 40:1 effector to target cell ratio, CIK cells killed 61.2% of the target tumor cells (59%-75.2%) and 82% (69.7%-90.4%), respectively. Three patients with refractory NHL were enrolled in our clinical protocol. 2 patients are male, one female, median age was 51.1 years (31.11-78.8); Karnofsky score was 70 or above. They received 3 cycles of escalated doses of CIK cells and a total of 9 infusions were administered. Each cycle consisted of 2-5 intravenous infusions of CIK cells on consecutive days, followed by the next cycles of infusions after 3 weeks. The number of transferred CD3+ cells per patient were 5-19×10⁶ in one course of treatment. The absolute number of CD3+CD56+ cells infused ranged from 2.5×10⁶ to 3.9×10⁶. Patients were monitored before, during and after treatment. One patient during the first cycle of infusions developed WHO grade 2 fever resolved with the addition of antibiotics. Sterility controls of CIK cells and blood cultures were negative. There were no other immediate adverse reactions to the infusions. The subjective symptom score remarkable improved in all the patients and the treatment showed to have a positive impact on quality of life. Conclusion: These results demonstrate that a sufficient number of CIK cells with potent in vitro cytotoxicity can be expanded from heavily pre-treated patients with advanced lymphoma. Interestingly, an initially bcl-2 overexpression, observed in 3 of the samples, was no longer detectable following extended culture suggesting that CIK cells may be derived from normal CD3+ precursor cells. Our preliminary results showed that feasibility and protocol adherence were excellent and the toxicity profile was favorable. Its potential clinical benefits are still under investigation and further studies are required to draw definite conclusions.

PU411
MULTIPARAMETRIC FOUR COLOUR FLOW CYTOMETRIC ANALYSIS OF HUMAN BONE MARROW-DERIVED MESENCHYMAL CELLS FROM NORMAL AND HEMATOLOGICAL PATIENTS
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There has been an increasing interest in the stromal system as subject of investigation in tissue engineering, cell transplantation, hemopoietic stem cell transplantation and gene therapy. There are no universally accepted antigenic determinants for the phenotypic characterization of bone marrow mesenchymal stem cells (MSC); the flow cytometric approach was based on single staining procedure. The purpose of this study was to utilise a cytofluorimetric multiparametric four colour panel based on the expression of CD105, CD90, CD106, CD59, CD36, CD31, CD166, CD34 antigens to study the antigenic expression patterns of human bone marrow derived cultured mesenchymal cells from patients with different hematological malignancies such as acute myeloid leukemia (8 patients), acute lymphoblastic leukemia (3), non Hodgkin’s Lymphoma (6), myelodys-
plastic syndrome (13) and 6 normal subjects. Our multiparametric analysis was based on forward- and side scattering properties as well as on four colour determination of cultured mesenchimal cells. The number of contaminating CD45+ cells, that are CD14+ CD11c+ or CD3+ cells, was found to be inversely correlated to the age of the stromal layers in all pathologies except for myeloma patients in which a consistent percentage of hemopoietic cells were also detected after 50-60 days of culture. In this study, mesenchymal cells were gated on CD45- 7AAD- events and then evaluated for the expression of different markers. The results showed that all mesenchymal cells are negative for CD34, CD31, CD36 antigen while are homogeneously positive for CD90, CD105, CD59. CD106+, CD45- 7AAD- cells were expressed in a low percentage of mesenchimal cultured cells. The CD45-7AAD-CD105+CD90+ phenotype was the most homogeneously represented cell subpopulations among the different pathologies examined as well as the healthy subject group. On the basis of these findings, whole fresh bone marrow samples from normal subjects before the culture was studied with these four color multiparametric flow cytometric staining. The results show a good correlation (r=0.83; Pearson test) between the in vitro culture clonogenic output (number of colony-forming unit fibroblasts, CFU-F/10^6 cells) and the expression of CD45- 7AAD-CD105+CD90+ cells subset. These findings would be of clinical interest during selection procedures before ex vivo expansion and therapeutic manipulation.