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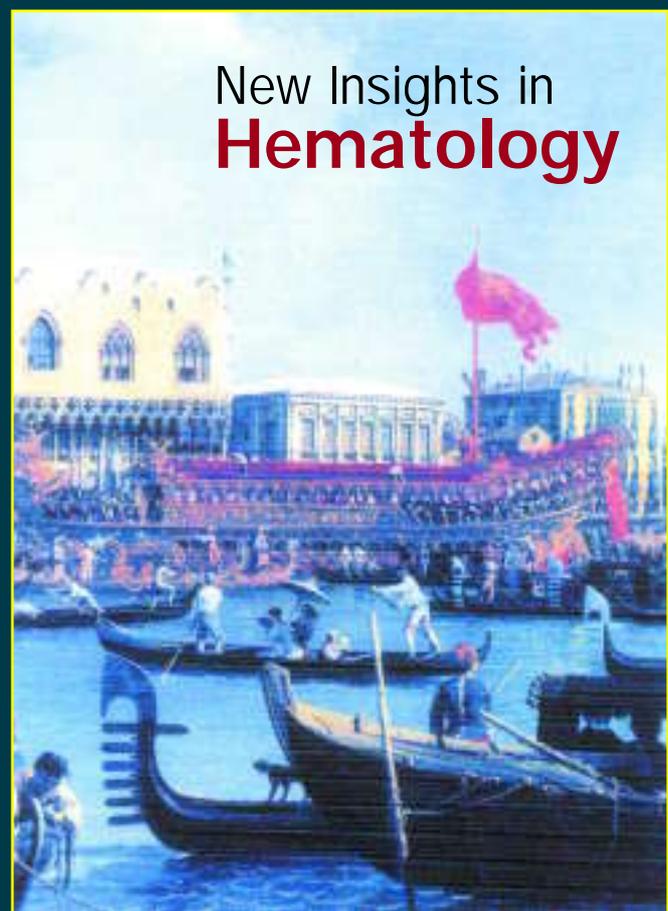
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NEW INSIGHTS IN HEMATOLOGY

President: Prof. Teodoro Chisesi

Venice, Italy
July 9-12, 2000



New Insights in
Hematology

New Insights in **Hematology**

Venice, Italy July 9-12, 2000

President: prof. Teodoro Chisesi



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SCIENTIFIC PROGRAM



Sunday, July 9th

Scuola Grande S. Giovanni Evangelista

6:00 p.m. **Opening Ceremony**

6:30 p.m. **Opening Lecture**

7:00 p.m. **Welcome Cocktail**



Monday, July 10th

9:00-10:30 a.m.

1st Session

HODGKIN'S DISEASE
(G. CANELLOS - R.T. HOPPE)

- | | |
|---|---------------------|
| 9:00 a.m. Dual transplantation (autograft followed by mini-allograft) for high-risk lymphomas » | A.M. Carella |
| 9:20 a.m. New insights in the classification » | F. Facchetti |
| 9:40 a.m. Primary therapy for advanced Hodgkin's Disease »» | A. Engert |
| 10:00 a.m. Hodgkin's Disease: the management of early stage disease » | R.T. Hoppe |
| 10:20 a.m. The management of the relapse » | G. Canellos |
| 10:30-11:00 a.m. Break | |

11:00 a.m. - 1:00 p.m.

2nd Session

THE NEW ENTITIES
(W. HIDDEMANN - P.G. ISAACSON)

- | | |
|---|----------------------|
| 11:00 a.m. New insights into the pathology of low grade-lymphoma » | P.G. Isaacson |
| 11:20 a.m. Malt lymphomas » | E. Zucca |
| 11:40 a.m. Mantle cell lymphomas » | W. Hiddeman |
| 12:00 a.m. Stage modified international prognostic index correlates to response rate and survival in patients with localized primary gastrointestinal diffuse large B-cell lymphoma » | S. Cortelazzo |
| 12:20 p.m. Treatment of cutaneous T-cell lymphoma (mycosis fungoides) » | D.J. Straus |
| 12:40 p.m. Primary effusion lymphoma (PEL): five years after its discovery » | A. Carbone |
| 1:00-2:00 p.m. Lunch | |

2:00-3:00 p.m.

Round Table

THROMBOEMBOLISM IN ONCOHEMATOLOGY

(A. GIROLAMI)

- ❖ Thrombosis in pediatric oncohematology » *C. Messina*
- ❖ Thrombosis and myeloproliferative diseases » *M.L. Randi*
- ❖ Antithrombotic drugs in oncohematology » *G. Cella*

2:30-4:30 p.m. **SLIDE SEMINAR FOR PATHOLOGISTS**

3:00-4:00 p.m.

THE BURNING QUESTIONS: THE THERAPY OF CLL

- 3:00 p.m. **Introduction: Reassessment of therapeutic goals in chronic lymphocytic leukemia: chemotherapy or hematopoietic stem cell transplantation for some patients? »** *A. Polliack*

Discussants

- 3:20 p.m. CLL treatment-chemotherapy» *E. Montserrat*
or
3:40 p.m. Transplant » *I. Majolino*

4:00 - 6:30 p.m.

3rd Session

NEW INSIGHTS IN PATHOLOGY/BIOLOGY

(P.G. ISAACSON - L. CHIECO-BIANCHI)

- 4:00 p.m. Etiology virus and oncogens » *L. Chieco-Bianchi*
- 4:20 p.m. New insights into the Classification of Non-Hodgkin's Lymphoma » *P.G. Isaacson*
- 4:40 p.m. Lymphoma phenotyping: new molecules of interest » *S.A. Pileri*
- 5:00 p.m. Diagnostic implications of genetic lesions in Non-Hodgkin's Lymphoma » *G. Gaidano*
- 5:20 p.m. The evaluation of response » *G. Saglio*
- 5:40 p.m. New biological prognostic factors in Hodgkin's Disease » *G. Pizzolo*
- 6:00 p.m. New perspectives in diagnosis of lymphoproliferative disorders » *G. Semenzato*



Tuesday, July 11th

8:30-10:30 a.m.

4th Session

NON HODGKIN'S LYMPHOMAS

(S. TURA - S. HORNING)

- 8:30 a.m. Therapy for diffuse aggressive lymphoma » **S. Horning**
- 8:50 a.m. Aggressive Non-Hodgkin's lymphoma:
is age still a limit to treatment? » **P.L. Zinzani**
- 9:10 a.m. Treatment of aggressive Non-Hodgkin's lymphomas according
to prognostic factors. Lessons from the GISL LA03 study » **M. Federico**
- 9:30 a.m. Conventional treatment of follicular lymphomas » **P. Solal-Céligny**
- 9:50 a.m. HIV-related Non-Hodgkin's lymphoma (NHL-HIV): general
characteristics and results of treatment
adapted to prognostic factors » **U. Tirelli**
- 10:10 a.m. Geriatric assessment in the management of elderly patients
with Non-Hodgkin's lymphoma (NHL) » **S. Monfardini**
- 10:30-11:00 a.m. Break

11:00 a.m. - 1:00 p.m.

5th Session

HIGH DOSE CHEMOTHERAPY

(A.H. GOLDSTONE - V. RIZZOLI)

- 11:00 a.m. ABMT in low grade » **J. Apostolidis**
- 11:10 a.m. Detection of minimal residual disease in hematological
malignancies after high dose chemotherapy » **V. Rizzoli**
- 11:30 a.m. High-dose sequential (HDS) chemotherapy: a feasible and effective
regimen for high risk Non-Hodgkin's lymphoma » **C. Tarella**

12:10 p.m. Allografting for high-risk patients: reduced intensity chemotherapy with thiotepa, fludarabine and cyclophosphamide allows a stable engraftment with low toxicity » **P. Corradini**

12:30 p.m. New roles for allogeneic transplantation in lymphoma » **A.H. Goldstone**

1:00-2:00 p.m. Lunch

2:00-3:30 p.m. **MAIN SYMPOSIUM: Rituximab**
(T. BARBU)

2:00 p.m. Molecular eradication of bcl-2/IgH chimeric gene in follicular Non-Hodgkin's lymphoma patients after therapy with CHOP and rituximab » **A. Rambaldi**

2:20 p.m. Molecular responses with Rituximab in newly treated Non-Hodgkin's lymphoma with a low tumor burden » **G. Salles**

2:40 p.m. Pilot trial of Rituximab and chemotherapy with infusional cyclophosphamide, doxorubicin and etoposide (CDE) in HIV-associated Non-Hodgkin's lymphoma » **U. Tirelli**

3:10 p.m. Rituximab in autoimmune diseases » **M. Baccarani**

3:30-4:30 p.m. **SATELLITE SYMPOSIUM:**
Erythropoietin α in the treatment
of hematological malignancies
(T. CHISESI - F. DAMMACCO)

3:30 p.m. Erythropoietin in hematological tumors » **G. Castoldi**

3:50 p.m. A placebo-controlled study on the effect of epoetin alpha in patients with multiple myeloma » **F. Dammacco**

4:10 p.m. Growth factors in haemopoietic progenitor cells mobilization » **P. Leoni**

4:30-6:00 p.m. **SELECTED ORAL PRESENTATIONS**
(G. SANTINI - D.J. STRAUS)

Determination of the drugs resistance of patients with malignant lymphoma in vitro » **Y. Chervonobab**

CD38 expression predicts poor clinico-biological features in B-cell chronic lymphocytic leukemia » **G. D'Arena**

Evaluation of Minimal Residual Disease (MRD) in low grade B-NHL: prognostic significance and technical problems. A single centre experience » **M. Riccardi**

- Impaired phagocytic function of polymorphonuclear neutrophils (PMN) in B lymphocytic leukemia (B-CLL) » **N. Porakishvili**
- Fludarabine in combination therapy is an effective treatment for relapsed or refractor low-grade non-Hodgkin's lymphoma: a multicenter experience » **A.M. Congiu**
- Cis-platinum, idarubicin, prednisone (CIP) as consolidation therapy after P-vabec chemotherapy for elderly patients with diffuse large lymphomas. An Italian multicenter randomized study » **M. Martelli**
- EBV-negative lymphoproliferative disorders in long term survivors after heart, kidney and liver transplant » **G. Dotti**
- Fludarabine plus cyclophosphamide is an effective regimen for patients with pretreated chronic lymphocytic leukaemia » **M. Spriano**
- A new purging strategy: "ex-vivo" treatment by Mabthera (anti-CD20) of PBSC graft and "in vivo" purging of B-CLL patients » **S. Volpe**
- HCV-associated non Hodgkin lymphoma/leukemias and malignant immunosecretory disorders in a single haematological center in Rome: a clinico-pathological study of 1993-1999 cases » **M. Marino**

6:00-7:30 p.m. **THE BURNING QUESTIONS: CONVENTIONAL CHEMOTHERAPY OR HDS?**

- 6:00 p.m. **Introduction** » **G. Canellos**
- 6:20 p.m. **Chemotherapy** » **P. Solal-Céligny**
- 6:40 p.m. **Chemotherapy (CT) or High-Dose Therapy for aggressive Non-Hodgkin's lymphoma (NHL)?** » **G. Santini**
- 7:00 p.m. **ABMT** » **A.H. Goldstone**
- 7:20 p.m. **HDS** » **C. Tarella**



Wednesday, July 12th

8:30-11:30 a.m.

6th Session

NEW DRUGS IN HEMATOLOGY

(M.J. KEATING - M. BACCARANI)

- 8:30 a.m. Rationale in new drugs strategies » **M.J. Keating**
- 8:50 a.m. Amifostine » **A. Olivieri**
- 9:10 a.m. Treatment of chronic lymphocytic leukemia
with oral fludarabine phosphate » **M. Klein**
- 9:30 a.m. Campath » **G. Pangalis**
- 9:50 a.m. A review of Zevalin radioimmunotherapy (RIT) for B-cell
Non-Hodgkin's lymphoma (NHL) » **J.L. Murray**
- 10:10 a.m. Daunoxome (DNX). New perspectives in hematology » **M. Baccarani**
- 10:30 a.m. Break
- 10:45 a.m. Oxaliplatin in Non-Hodgkin's lymphoma (NHL):
a new therapeutic alternative? » **E. Cvitkovic**
- 11:05 a.m. Vinorelbin » **A. Santoro**

11:30 a.m. - 1:00 p.m.

7th Session

MULTIPLE MYELOMA

(M. BOCCADORO - R. BATAILLE)

- 11:30 a.m. Bone disease in myeloma » **R. Bataille**
- 11:50 a.m. Intensified chemotherapy for elderly patients » **M. Boccadoro**
- 12:10 a.m. AlloBMT » **I. Majolino**
- 12:30 a.m. Multiple myeloma: new frontiers in ABMT » **P. Coser**



1st session
HODGKIN'S DISEASE
(G. Canellos, R.T. Hoppe)

DUAL-TRANSPLANTATION (AUTOGRAFT FOLLOWED BY MINI-ALLOGRAFT) FOR HIGH-RISK LYMPHOMAS

Carella AM

Azienda Ospedale San Martino e Cliniche Universitarie Convenzionate, Genoa, Italy

The greater potential benefit of alloSCT could be exploited if conditioning mortality could be decreased and tumor burden minimized before conditioning. One method of achieving this would be to use high-dose therapy (HDT) and autoSCT to debulk lymphoma, followed by alloSCT using a nonmyeloablative conditioning regimen (NMR). Between June 1997 and October 1999, 19 patients with HD (n=13) and NHL (n=6) received this combined procedure. All patients had advanced/resistant disease. Two patients had already received a first autograft. Patients received cyclophosphamide (3 g/m²) and recombinant human granulocyte-colony stimulating factor to mobilize autologous hematopoietic stem cells. Subsequently, they received HDT [carmustine, etoposide, cytarabine, and melphalan] (BEAM protocol) and re-infusion of autologous stem cells. At a median of 50 days after engraftment, patients received fludarabine 30 mg/m² with cyclophosphamide 300 mg/m² daily for three days. Donor-mobilized hematopoietic stem cells collections were prepared for fresh infusion and were not T-cell depleted. Methotrexate and cyclosporine were used to prevent graft rejection and as GVHD prophylaxis. Patients with mixed chimerism or with complete chimerism and residual lymphoma, received donor lymphocyte infusion 60-300 days post-transplantation (if GVHD was not present). Complete (n=14) and mixed (n=5) chimerism was achieved in all patients. The median follow-up is of 325 days (range, 67-840 days) from mini-allografting. Fourteen patients are alive: 6 patients are in complete clinical remission and 5 patients are in good partial remission. Five patients died, three of progressive HD/NHL and two of progressive HD/NHL combined with extensive chronic GVHD. In conclusion, the Flucy protocol produces engraftment and generation of graft-versus-lymphoma in high-risk patients previously debulked with autografting.

PRIMARY THERAPY FOR ADVANCED HODGKIN'S DISEASE

Engert A, Diehl V

Clinic for Internal Medicine I, University of Cologne, Cologne, Germany

Advanced stage Hodgkin's disease (HD) was incurable until the introduction of polychemotherapy. With MOPP or similar regimens, complete remission rates of 73% to 81% and long-term overall survival of 50%

to 64% were obtained. Later, it was demonstrated that ABVD was non-cross resistant and had less acute and long-term toxicities. Large randomized trials demonstrated that results with the combination of MOPP/ABVD were better than with MOPP alone. Since ABVD alone was at least as effective as MOPP/ABVD in follow-up trials, 8 cycles of ABVD became regarded as the gold standard for the treatment of advanced HD by most groups. The role of radiotherapy in the additional treatment of initial bulky or slow responding areas is not precisely defined. More recently, a new drug combination, BEACOPP, was demonstrated by the German Hodgkin Study Group to give superior results to ABVD as far as response rates and freedom from treatment failure are concerned. Thus, BEACOPP might become the future standard regimen for the treatment of patients with advanced Hodgkin's disease.

HODGKIN'S DISEASE: THE MANAGEMENT OF EARLY STAGE DISEASE

Hoppe RT

Department of Radiation Oncology, Stanford University, CA, USA

Radiation therapy has a long history in the treatment of early stage Hodgkin's disease. By the late 1980s, the standard approach to management was to perform a staging laparotomy and, if negative, proceed with radiation to mantle and paraortic fields (STLI). The expected outcome at 10 years was a relapse-free rate of 75-80% and survival of 90%.

Challenges to this approach came from the EORTC. In the H5 study, patients with a negative staging laparotomy were randomized to receive mantle irradiation alone or STLI. At 15 years, there are no differences in survival or freedom from treatment failure. In a subsequent trial, patients were randomized to laparotomy. Patients who underwent laparotomy were treated according to the stage of disease and patients without laparotomy were treated with STLI/spleen fields. At 15 years, there is no difference in freedom from treatment failure, but a statistically significant difference in survival favoring no laparotomy. Based upon these clinical trials, the standard radiation treatment is with a mantle field alone after a negative laparotomy or STLI if a laparotomy is not performed. A final issue is whether both laparotomy and subdiaphragmatic treatment could be avoided in patients with the most favorable prognosis. In the EORTC H7VF trial, patients with the most favorable disease characteristics were treated with mantle irradiation alone, without laparotomy. The six-year event-free survival was only 66%. The conclusion was that this management approach was inadequate, even for patients with the most favorable prognostic factors.

Because of the late risks of radiation, especially solid tumors and cardiovascular disease, a good alternative is combined modality therapy (CMT). Early studies in the 1970s, combined MOPP with high dose irradiation. In general, CMT showed a superior event-free but similar overall survival compared to programs utilizing radiation therapy alone.

In the absence of a demonstrable survival advantage, CMT of early stage disease seemed inappropriate, since all patients were being exposed to the toxicities of both modalities. A major advance was the development of an effective chemotherapy regimen (ABVD) that was not associated with leukemia or sterility. A new effort was launched in trials of CMT to find an optimal combination of chemotherapy and irradiation. The thrust of the new programs was to treat with shorter courses of chemotherapy and follow with limited radiation. In Milan, patients were treated with four cycles of ABVD followed by involved or extended field irradiation. In a German Hodgkin's Study Group trial, patients were treated with four months of COPP/ABVD followed by involved or extended field irradiation. The results of both trials were excellent. Current clinical trials for CMT attempt to define the least amount of chemotherapy and irradiation to achieve reliable cure rates. An EORTC trial employs six months of a "mild" chemotherapy, EBVP I, and randomizes the radiation dose. A GHSG trial includes a double randomization: two versus four cycles of ABVD and two different doses of irradiation. In a pilot study at Stanford, patients are treated with eight weeks of Stanford V chemotherapy followed by involved field irradiation. Long term assessment of outcomes and toxicity will be required to define the best CMT regimen for the majority of patients.

MANAGEMENT OF RELAPSED HODGKIN'S DISEASE

Canellos GP

Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA, USA

Relapse of localized or advanced Hodgkin's disease occurs in a significant minority (20-35%) of patients. The overall survival of any institutional series approximates 75%, but almost one quarter of patients will have a recurrence that has the potential to be fatal. Relapse is usually proportional to unfavorable prognostic features, for both localized and advanced disease. The prognostic features that predict good response to primary treatment generally correlate with the likelihood of benefit of second-line or salvage therapy. In the vast majority of cases, relapse will be treated by systemic treatment. In rare examples of isolated nodal relapse following chemotherapy alone, patients can be treated with localized radiation therapy that cures the relapse. However, in most instances, combined modality or systemic therapy alone is required.

The persistence of residual radiographic masses as a reason for concern has decreased with the expanded knowledge that a residual mass can regress in time. The availability of ⁶⁷Ga gallium nuclear scans, PET scans and the general principle of close follow-up in a stable partial remission has decreased the amount of unnecessary second-line therapy in the mistaken impression that residual masses represent active disease. In patients who receive radiation therapy only, relapse – if it were to occur – would be in previously unirradiated areas whereas in patients treated with systemic therapy alone recurrences generally occur in

areas of prior known disease. Similarly, when combined modality therapy is used, disease would be more likely to recur in unirradiated areas. Most patients who have recurrence following radiation therapy alone can be successfully treated with systemic therapy, and 2/3 to 3/4 of patients can be salvaged by systemic treatment – the 10-year survival post relapse is approximately 60%. Relapse from systemic treatment occurs in approximately 30-35% of patients treated with any regimen, including ABVD. About 1/3 of the failures will, in fact, be progressive or refractory disease with the remainder being divided between those whose remission lasts less than 12 months (33-40%) and those whose remission lasts longer than 12 months (~25%). The 12-month period has been employed as an arbitrary interval with relapses less than 12 months generally characterized by being more aggressive and less responsive to second-line therapy, often requiring more intensive treatment. Late relapse, on the other hand, that is localized to nodes has a particularly high second line salvage rate (65-80% if the disease is confined to lymph nodes). Conventional dose chemotherapy salvage treatment for patients who fail in less than 12 months produces a failure-free survival limited to 15% whereas conventional dose salvage is much higher (35-50%) in those who relapse beyond 12 months. High-dose therapy with either peripheral stem cells or bone marrow is used as a salvage modality for induction failures – in this setting the results of high-dose therapy are in the range of 30% of patients being relapse-free at five years. However, in patients with favorable characteristics who relapse beyond 12 months, the advantage of using high-dose therapy over conventional-dose salvage is questionable. For the patients who relapse after standard chemotherapy, there is no ideal second or third line program. The additional therapy given in salvage treatment often entails the use of alkylating agents which, in turn, will increase the risk of alkylating agent-induced bone marrow damage, from which the patient is spared by the use of ABVD and related regimens.

2nd session
THE NEW ENTITIES
(W. Hiddemann, P.G. Isaacson)

NEW INSIGHTS INTO THE PATHOLOGY OF LOW-GRADE LYMPHOMAS

Isaacson PG

Department of Histopathology, University College London Medical School, University Street, London, UK

The term *grade* when used to qualify a lymphoma refers to the cytological properties of the neoplastic cells and not to clinical behavior. Lymphomas comprised by small cells with nuclei containing dense chromatin, usually showing a low proliferation fraction are described as *low-grade*. These include a morphologically diverse group of diseases with marked

differences in clinical behavior that require markedly different treatments. Included in this group are lymphocytic lymphoma (chronic lymphocytic leukemia), prolymphocytic leukemia, hairy cell leukemia, follicular lymphoma, mantle cell lymphoma, marginal zone lymphomas of extranodal (MALT) type and nodal type and splenic marginal zone lymphoma. New insights into molecular biology have made a major contribution to the identification of these separate diseases and in many cases have led to the development of new markers that identify their characteristic immunophenotypes. In this respect, the use of antibodies to BCL-2 protein and cyclin D1 has greatly facilitated the differential diagnosis of mantle cell and follicular lymphoma and similar progress is likely in the near future for MALT and splenic marginal zone lymphomas. The relationship of many of these "low-grade" lymphomas to the B-cell follicle has been underlined by their tendency to manifest a follicular growth pattern which can in itself lead to problems with differential diagnosis.

MALT LYMPHOMAS

Zucca E, Cavalli F

Oncology Institute of Southern Switzerland, Ospedale San Giovanni, Bellinzona, Switzerland

Far from being a rare entity, extranodal marginal zone B-cell lymphomas of MALT type account for about 7% of non-Hodgkin's lymphomas. The pathologic features of MALT lymphomas are similar regardless of their site of origin but they have been best characterized in gastric lymphomas. Some histological features suggest that the lymphoma cells may participate in an immune response. These include the presence of plasma cells, scattered transformed blasts, reactive non-neoplastic lymphatic follicles and reactive T-lymphocytes. The onset of MALT lymphoma in the stomach is preceded by the acquisition of MALT as a result of *H. pylori* infection. The micro-organism, which is known to be responsible for chronic gastritis, can be found in the gastric mucosa in nearly all instances of gastric MALT lymphoma, with several lines of evidence suggesting a link between *H. pylori*-chronic gastritis and the lymphoma. Indeed, the presence of the B-cell clone that subsequently becomes predominant in the transformation to MALT lymphoma has been demonstrated in *H. pylori* gastritis specimens taken several years before development of the lymphoma. The prognosis of early, i.e. localized, gastric MALT lymphoma is excellent regardless of the treatment employed. The link of the lymphoma with *H. pylori* infection has led to investigations of the effect on the lymphoma of eradicating the micro-organism. Antibiotics can be employed effectively as the sole initial treatment, although, it is still unknown whether *H. pylori* eradication will definitely cure the lymphoma. Long-term follow-up of antibiotic-treated patients is, therefore, mandatory. The efficacy of antibiotic therapy is reduced in locally advanced disease, with bulky masses or deep infiltration of the gastric wall, in the cases without evidence of *H. pylori* infection and in disease associated with increased numbers of large cells.

No treatment guidelines exist for the management of patients after antibiotics have failed and a choice can be made between conventional oncological modalities; chemotherapy or radiotherapy can be effective and the role of surgery should be redefined. The experience with MALT lymphoma arising outside the stomach is limited because no institution can collect series of patients with adequate numbers of cases for each given localization. The choice between surgery, irradiation and chemotherapy will depend on the patients' clinical conditions, and on the tumor location and extension.

MANTLE CELL LYMPHOMAS

Hiddemann W, Dreyling M

Dept. of Hematology/Oncology, University of Munich, Germany

Mantle cell lymphoma (MCL) represents a recently broadly accepted lymphoma subtype that accounts for approximately 5-8 % of all lymphomas. The disease occurs in elderly patients and affects predominantly the male sex. The translocation t(11;14) is thought to be of major importance in the pathogenesis of MCL. By this abnormality the cyclin D1 gene is juxtaposed to the gene of the immunoglobulin heavy chain which leads to an overexpression of cyclin D1 and deregulation of the cell cycle. Based on histology and cytology two major types of MCL can be discriminated: typical MCL and variant MCL of the blastoid subtype. This latter subgroup is characterized by a high mitotic rate and Ki 67 expression and frequently involves p53 mutations. The clinical course is unfavorable.

The treatment of MCL remains a dilemma in spite of numerous efforts to develop more effective and intensive regimens. A high proportion of cases respond to initial cytoreductive therapy with partial or complete remission which is, however, of short duration. The median survival of patients is in the range of only 3-4 years.

New perspectives may arise from myeloablative radiochemotherapy followed by blood stem cell transplantation or from non-myeloablative allogeneic transplantation. In addition anti-lymphoma immunotherapy may alter the unfavorable outcome and may offer new therapeutic strategies.

STAGE-MODIFIED INTERNATIONAL PROGNOSTIC INDEX CORRELATES TO RESPONSE RATE AND SURVIVAL IN PATIENTS WITH LOCALIZED PRIMARY GASTROINTESTINAL DIFFUSE LARGE B-CELL LYMPHOMA

Cortelazzo S on behalf of the International Extranodal Lymphoma Study Group (IELSG)

Divisione di Ematologia, Ospedali Riuniti, Bergamo, Italy

The definition of prognostic parameters in early stages of primary gastrointestinal lymphoma (PGIL) is still controversial.

The aim of this retrospective analysis was to assess the value of a prognostic model based on a stage-modified International Prognostic Index (IPI) (Miller

et al., 1998), in which the *Lugano staging system for GI lymphomas* (Rohatiner *et al.*, 1994) replaced the Ann Arbor staging system. The model included 3 risk categories (low, intermediate, high) defined on the basis of the number of risk factors (0-1, 2, ≥ 3).

From April 1972 to December 1998, 547 consecutive, unselected patients, 435 with gastric lymphoma (GL) and 112 with intestinal lymphoma (IL) from 7 Italian, 1 Greek and 1 Swiss centers had a diagnosis of localized diffuse large B-cell histology (WHO, 1999) PGIL. Patients with IL were slightly younger than those with GL (age >60 years= 41% vs 51%; $p=0.08$), but had more risk factors such as Lugano staging \geq II2 (49% vs 28%; $p=0.0001$), elevated LDH (28% vs 17%; $p=0.01$) and poor ECOG-performance score (39% vs 17%; $p=0.0001$). Therefore, more IL than GL patients belonged to the high risk category (24% vs 12%; $p=0.004$). The percentage of patients who were given single or combined first treatment was comparable in the GL and IL groups (32% vs 24% and 68% vs 76%, respectively) and the prevailing therapy was surgery followed by chemotherapy (GL=48% and IL=70%). The complete remission (CR) rate and the percentage of patients alive in first continuous CR (CCR) were significantly superior in patients with gastric than in those with intestinal lymphoma (91% vs 74%; $p<0.001$ and 66% vs 53%; $p=0.01$, respectively). In both groups CCR depended on risk category, ranging from 77% to 33% in GL and from 65% to 23% in IL patients. The five-year estimate of overall survival (OS) was better in GL than in IL patients (75% vs 63%, $p=0.04$). Also the five-year estimate of OS varied according to risk categories, ranging from 87% to 40% ($p<0.001$) in GL patients and from 77% to 27% ($p<0.001$) in IL patients. Moreover, in each risk category, OS of patients who were given combined treatment was superior to that obtained with single therapy. Univariate analysis showed that intermediate and high risk categories, bulky disease, B symptoms and intestinal presentation were related to a poor outcome, but by Cox multivariate analysis intermediate and high risk categories were the strongest predictors of survival (RR=2.32, 95% CI: 1.5-3.5, $p=0.0001$ and RR=5.95, 95% CI: 4.0-8.8; $p=0.0001$, respectively). The proposed prognostic indicators of PGIL allow identification of subgroups with significantly different response rates to therapy and survival. The poorer outcome of IL patients in comparison with that of patients with gastric localization was correlated with the more frequent adverse presentation as determined by our model. However, this prognostic model should be validated in future prospective clinical trials.

TREATMENT OF CUTANEOUS T-CELL LYMPHOMA (MYCOSIS FUNGOIDES)

Straus DJ

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The classical treatment for limited mycosis fungoides is topical chemotherapy with nitrogen mustard or carmustine (BCNU) or high-potency steroids (betamethasone). Another popular treatment is 8-methoxypsoralen, a compound that interferes with

DNA and RNA synthesis when activated by long wavelength ultraviolet light A (PUVA). The third classical treatment is with radiation therapy which can be delivered superficially to the entire skin using electrons (total skin electron beam radiation therapy). Cutaneous T-cell lymphomas (CTCL), unless localized and excised completely or irradiated, are difficult to cure, although long-term durable remissions have been reported in a minority of patients with limited mycosis fungoides treated with topical nitrogen mustard or total skin electron beam radiation therapy. The systemic treatments of patients with more advanced disease are not curative. Methotrexate is one of the most active chemotherapeutic agents and others include alkylating agents (nitrogen mustard, cyclophosphamide, chlorambucil), antibiotics (doxorubicin, bleomycin), vinca alkaloids (vincristine, vinblastine), epipodophyllotoxins (etoposide), purine analogs (cladribine, pentostatin) and various combinations of these drugs. Interferon α -2a produced partial responses in approximately 50% and complete responses in approximately 15% of patients with mycosis fungoides. The best treatment dose schedule is not completely established but a reasonable starting dose is 3 million units subcutaneously daily or 3-6 million units three times per week. Excellent results in refractory mycosis fungoides were recently reported with a combination of PUVA and interferon α -2a. A novel immunotherapy treatment for CTCL has been recently introduced with an immunotoxin recombinant DNA-derived fusion protein composed of the amino acid sequences for diphtheria toxin fragments A and B and sequences for interleukin-2 (DAB₃₈₉IL-2, denileukin diftitox, Ontak®). In approximately two thirds of cases of CTCL, the lymphoma cells express the IL-2 receptor on their surface. The high-affinity IL-2 receptor (CD25/CD122/CD132) is the target for the immunotoxin that results in decreased cellular protein synthesis and cell death caused by the diphtheria toxin. In the pivotal trial 71 patients with CTCL were randomized to DAB₃₈₉IL-2, either 9 μ g/kg/day or 18 μ g/kg/day i.v. over 30-45 min. for five days every 22 days for up to 11 courses. The overall response rate was 30%. There was not a significant difference in response between patients in the 2 arms, although the response rate was higher for patients with advanced stages (\geq IIB) treated with the 18 μ g/kg/day dose. The retinoids, analogs of vitamin A, are well known to inhibit cell proliferation and promote cell differentiation in both epithelial and non-epithelial tissues. They have long been used for the treatment and prevention of several skin diseases including psoriasis, acne, lichen planus, actinic keratosis and keratoacanthoma. Isotretinoin (13-cis-retinoic acid) has been used most frequently for mycosis fungoides in doses of 0.1-0.2 mg/kg/day with responses in approximately 40% of patients. Etretinate has also been used in similar doses. Bexarotene (Targretin®) is a synthetic retinoid that selectively activates retinoid X receptors (RXRs). These retinoid receptors have biologic activity distinct from that of retinoic acid receptors (RARs). Once activated, these receptors function as transcription factors that regulate the expression of genes that control cellular differentiation and proliferation.

By mechanisms that are not known, activity has been found against CTCL, and the drug has recently been released commercially. The overall response rate in patients with refractory or persistent early stage CTCL was 48% and 49% in patients with refractory advanced stage disease. The optimal dose was 300 mg/m²/day. Patients on the trials were treated for up to 97 weeks. New biological approaches may improve the outlook for CTCL.

PRIMARY EFFUSION LYMPHOMA: FIVE YEARS AFTER ITS DISCOVERY

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Primary effusion lymphoma (PEL) is a B-cell neoplasm characterized by infection of the tumor clone by human herpesvirus type-8/Kaposi's sarcoma-associated herpesvirus (HHV-8/KSHV) and by liquid growth in the serous body cavities with no formation of solid tumor masses. Throughout its entire clinical course, the lymphoma tends to remain localized to the serous body cavities. The epidemiology of PEL points to a close link with underlying immunodeficiency of the host, since most cases develop in individuals severely immunocompromised because of pre-existing acquired immunodeficiency syndrome. The histogenesis and pathogenesis of PEL have been clarified by intensive investigations performed since the disease was recognized in 1995. PEL is composed of tumor cells which reflect post-germinal center B-cells; they morphologically bridge immunoblastic and anaplastic features, and phenotypically display a non-B, non-T phenotype consistent with late stages of B-cell differentiation. HHV-8/KSHV is thought to play a major role in the pathogenesis of PEL via expression of several viral latent genes which have the potential to affect B-cell growth. Other factors involved in PEL pathogenesis include cell cycle abnormalities and infection by Epstein-Barr virus, which occurs in 70 % of PEL cases. Five years after discovery of the disease, the distinctiveness of the biological and clinicopathologic features of PEL have prompted its recognition as an independent lymphoma category by the World Health Organization classification system of hematologic neoplasms.

Round Table THROMBOEMBOLISM IN ONCOHEMATOLOGY (A. Girolami)

VENO-OCCLUSIVE DISEASE OF THE LIVER AFTER PEDIATRIC BONE MARROW TRANSPLANTATION: A SINGLE CENTER EXPERIENCE

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Two hundred and nineteen children received a stem cell transplantation for the following underlying diseases: acute lymphoblastic leukemia (70), acute myeloid leukemia (44), malignant lymphomas (21), neuroblastoma (21), soft tissue sarcoma (22), other tumor (16), and non malignant diseases (25). The source of stem cells were: autologous (126), allogeneic matched sibling (56), unrelated donor (25), cord blood (6), haploidentical donors (4). The diagnosis of veno-occlusive disease (VOD) was made according to the Seattle criteria (jaundice, painful hepatomegaly or fluid retention). Twenty-nine of 219 patients (13.2%) developed clinical signs of VOD a median of 9.5 days (range 3-15) after infusion. Three patients were at their second transplant. According to prophylaxis, 11 out of 86 patients (12%) not receiving any drugs, 5 out of 26 (23%) receiving pentoxifylline, 6 out of 31 (19%) taking PGE₁, and 7 out of 76 (9.2%) treated with heparin developed VOD. The incidence in the allogeneic and autologous setting was 15% and 11.9%, respectively. Sixteen patients (55%) developed severe VOD and ultrasound studies showed inversion of portal venous flow. All patients received supportive therapies. Four patients died of VOD and none of them received any thrombolytic therapy. Twelve of 16 patients were treated as follows: 4 patients received PGE₁, a median of 5 days after inversion of portal venous flow; 2 of them did not show any benefit and were treated with recombinant tissue plasminogen activator (rTPA) 14 days after diagnosis; 4 patients were treated with rTPA ± heparin, starting on the day of flow inversion and all resolved; 4 patients received defibrotide beginning the day on which inversion was found; 2 of these cases resolved, and 2 were treated with rTPA. Overall, portal venous flow reverted to normal in 11/12 patients. Two of 12 patients (1 responder, 1 non-responder) died of multiorgan failure and sepsis. Gastro-intestinal hemorrhages were observed in 2 patients, both treated by rTPA as second line treatment.

In our experience, VOD mortality was 37% but the close monitoring of portal venous flow by ultrasound and early treatment with thrombolytic therapy seems to be very promising.

THROMBOSIS AND MYELOPROLIFERATIVE DISORDERS

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Polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) are the so called Philadelphia-negative myeloproliferative disorders (MPD) originating from a single neoplastic stem cell in the bone marrow. The clonal expansion leads to the proliferation of different hematologic cells with different phenotypic expression. MPD are generally diseases of middle or older age with a long life expectancy, in the case of ET similar to that of normal people.

Thrombotic and hemorrhagic events are typical complications in PV and ET patients, but thromboses are more frequent than hemorrhages and may be fatal or at least may induce permanent disability. While in PV, the rheological alterations may suggest a satisfactory explanation for the thrombotic events, in ET there is no correlation between the degree of thrombocytosis and thrombotic complications. However, lowering the platelet count in patients with MPD and active thrombotic complications may result in symptomatic improvement. In ET patients the only factors clearly associated with a higher risk of thrombosis are advanced age and a previous thrombotic complication. In different large series some correlation has been observed between thrombosis and smoke and/or hypercholesterolemia, at least in the cases who developed arterial thrombotic complications.

Thirty to fifty per cent of patients with MPD have microvascular occlusive or major vascular thromboses, which are most pronounced in elderly. Microvascular occlusive events can affect extremities with erythromelalgia, digital ischemia or burning pain which can progress to frank gangrene and necrosis of the digits. Arteriolar platelet thrombi are responsible for these symptoms and often they respond promptly to antiaggregating agents or lowering platelet drugs. The most common occlusive complications in PV and ET involve the cerebral circulation inducing neurologic manifestations such as non-specific headache, dizziness, transient ischemic attacks and stroke. Coronary, peripheral, and renal artery thromboses are also observed in these patients. In contrast, pulmonary embolism and deep vein thrombosis are only rarely reported in clinical studies. Venous thrombotic occlusions in unusual sites (portal vein system, hepatic veins, cerebral venous sinuses) are typical of young patients with ET and PV. Moreover, PV and ET are the cause of about two thirds of the cases of hepatic vein thrombosis, but this high prevalence has been recognized only since the identification of occult MPD with spontaneous erythroid colony formation in patients without significant changes in the peripheral blood. Multiple placental infarctions are considered the cause of recurrent abortion, fetal growth

retardation and premature deliveries which are frequently described to complicate the pregnancies in women with ET.

While in PV the blood volume should be normalized as rapidly as clinically possible to avoid the risk of vascular occlusion, the indications for reduction of the platelet count in ET remain disputed because many individuals are asymptomatic at presentation but also remain free of hemostatic complications during long-term follow-up. Low-dose aspirin seems to represent a safe and efficient treatment preventing for vascular occlusion in ET patients. However, prospective randomized trials are needed to assess the risk/benefit ratio of such a therapy.

ANTITHROMBOTIC DRUGS IN ONCOHEMATOLOGY

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Thromboembolic complications have been clearly associated with cancer. However in oncohematology only patients affected by chronic myeloproliferative disease or acute promyelocytic leukemia have increased risk of developing thrombosis. In contrast the prevalence of deep vein thrombosis (DVT) in lymphoproliferative disorders seems to be lower. Bone marrow transplantation is also associated with thrombotic complications such as veno-occlusive disease.

Aspirin is widely used in patients with thrombocythemia and polycythemia vera to prevent thrombotic complications. However, uncertainty still exists as to the benefit/risk ratio of aspirin prophylaxis in this setting. The ECLAP study is a randomized trial designed to clarify whether low dose aspirin is safe and superior to placebo in patients affected by polycythemia vera.

The management of DVT in oncohematology patients, due their serious underlying condition, is often difficult. Heparin remains the anticoagulant of choice to treat acute thrombotic episodes. However, potentially severe, although rare, side effects such as bleeding, acute anaphylaxis or heparin-induced thrombocytopenia can develop. More recently, with the introduction of low molecular weight heparin, these side effects seem to have been reduced so that this anticoagulant appears safer and effective.

There are, however, other potentially safe and effective compounds. Defibrotide is a polyelectrolyte drug similar to heparin. In contrast to heparin, its charge is due to the presence of multiple phosphate groups. Among others antithrombotic activities such as the increase of tissue plasminogen activator and protein C and expression of thrombomodulin, it is able to induce the release of tissue factor pathway inhibitor from the endothelial cells. Recently, given intravenously, it has been shown to be effective in critically ill patients for severe hepatic veno-occlusive disease after stem cell transplantation with the resolution of VOD in 40% of cases.

The burning questions:
THE THERAPY OF CLL

REASSESSMENT OF THERAPEUTIC GOALS IN CHRONIC LYMPHOCYTIC LEUKEMIA: CHEMOTHERAPY OR HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR SOME PATIENTS?

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Chronic lymphocytic leukemia (CLL) is still essentially an incurable disease when conventional chemotherapy regimens are used. However a small proportion of patients may be able to be "cured" after successful allogeneic stem cell transplantation. In general, it can be concluded that in recent years and during the last decade in particular, there have been a number of advances in therapy, related to the development of new drugs, introduced as part of the new perspectives for treatment of CLL. In "younger" patients (<65 years of age) in a higher clinical risk category a "curative-intent" approach has been entertained in some patients, using novel agents particularly the purine analogs (fludarabine, cladribine) with or without the addition of monoclonal antibodies such as Campath 1-H, and anti-CD-20, rituximab, and even radiolabeled anti-B-cell antibodies have been introduced into clinical trials very recently. The incidences of a good partial remission (PR) and complete remission (CR) have increased with the use of these regimens, some of which have been tested in large multi-institutional randomized studies. Although there is an impressive improvement in PR and CR rates and durable remissions are obtained in patients receiving the purine analogs and monoclonal antibodies compared with patients receiving chlorambucil or CHOP/CAP regimens, a convincing increase in survival for the group treated with the purine analogs has not been demonstrated. Nevertheless, because of the above CR/PR data, purine analogs are being increasingly used as the drugs of choice for initial treatment in "younger" age groups, particularly if the therapeutic goal is to achieve meaningful remission and to continue to utilize this window of opportunity to treat patients with curative intent employing a subsequent stem cell transplantation. Transplant procedures are indeed options to be considered in this subset of younger patients, particularly in those with poor prognostic features, regarded as a higher risk category of CLL, who will not be candidates for the usual "wait and watch" policy. There are indeed well defined, predictable prognostic features based on clinical, laboratory, biological, immunophenotypic and molecular genetic

findings which can perhaps select out CLL patients with a higher risk of progression and shorter survival. It is in this group of patients that more innovative therapeutic approaches including transplantation should perhaps be considered. This session will deal with these subgroups of CLL patients and discuss whether chemo-immunotherapy is more advantageous than standard therapy and whether transplant should be considered as part of the standard therapeutic approach for these individuals. However, notwithstanding the possible conclusions of this debate, it is obvious to all clinicians involved in this rapidly changing field of CLL that controlled clinical trials comparing the different therapeutic options must be performed before the true value of stem cell transplantation in CLL can be correctly assessed.

TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH CHEMOTHERAPY

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Chemotherapy continues to be the mainstay of treatment for chronic lymphocytic leukemia (CLL). For many years, CLL treatment revolved around the use of chlorambucil. The promising results observed in the 1980s with combination chemotherapy regimens such as COP, CAP, CHOP or mini-CHOP were not confirmed in randomized trials. Thus, while the response rate was found to be higher with combination chemotherapy than with chlorambucil, this did not translate into a longer survival. More recently, purine analogs – particularly fludarabine and 2-chlorodeoxyadenosine – have shown to be the most effective agents in the treatment of CLL. However, randomized trials have shown no survival advantage of patients treated with purine analogs as compared to those receiving chlorambucil followed by purine analogs in case of failure or progression. This might lead to a false sense of stagnation in the treatment of CLL with chemotherapy. However, progress has been made and is likely to continue. The reasons for this assertion are the following: 1) the response rate, including complete responses, obtained with purine analogs is the highest ever observed with single agents in CLL; 2) purine analogs prolong the disease-free interval; 3) purine analogs have synergism with other agents such as cyclophosphamide; results of ongoing trials with purine analog-containing regimens show impressive response rates, including "clonal" responses; 4) preliminary results from more immature trials combining chemotherapy and monoclonal antibodies are extremely promising. Ultimately, all these efforts should lead to improvement not only the response rate but also the quality of the responses, a necessary condition to envisage a cure for this disease.

3rd session
NEW INSIGHTS IN PATHOLOGY/BIOLOGY
(P.G. Isaacson, L. Chieco-Bianchi)

NEW INSIGHTS INTO THE CLASSIFICATION OF NON-HODGKIN'S LYMPHOMA

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The first clinically relevant lymphoma classification was proposed by Rappaport in 1966. The realization of the importance of the follicle center in lymphocyte proliferation and its relevance to lymphoma together with the discovery of B and T-lymphocytes provoked a number of new lymphoma classifications, including the Kiel classification. Attempts to resolve frustrating differences in emphasis and terminology between these classifications led to the emergence of The Working Formulation For Clinical Usage as a *translation system* between the different classifications. Consequently, the Working Formulation and the Kiel classification became the preferred classifications of NHL in the USA and Europe respectively. Trans-Atlantic divisions in lymphoma classification became increasingly institutionalized and in this setting the international lymphoma study group (ILSG) proposed the Revised European American Lymphoma (REAL) Classification. The basis for this new classification was a list of well defined (real) entities that were clearly recognized by all 19 ILSG members. The principles underlying the definition of these entities were primarily morphologic but included, immunophenotypic and genetic properties, the normal cell counterpart if known, and clinical features. To lend order to the classification, entities were subdivided according to their expression of B- and T (or NK)-cell surface markers and their origin from precursor or peripheral cells. Histologic grade as distinct from clinical aggressiveness was taken into account but not used as a primary defining feature. Both the clinical relevance and reproducibility of the REAL classification have been confirmed. Subsequently, the WHO has sponsored a new classification of all hematologic malignancies. The WHO NHL classification is, in effect, an updated REAL classification.

LYMPHOMA PHENOTYPING: NEW MOLECULES OF INTEREST

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Immunohistochemistry is an integral part of diagnosing malignant lymphomas. Besides defining the histogenesis, it allows the detection of molecules relevant to the prognosis and therapy of these disorders. In this light, several markers merit attention:

among them, the *bcl6* and *bcl2* oncogene products, IRF4, BSAP, CD10, and ALK protein. BSAP is the PAX-5 gene product, which is expressed at the nuclear level in all B-cell lymphomas including those derived from precursor elements. As go search for this, along with that of IRF4 – interferon regulatory factor-4, an indicator of plasma cell maturation – helps in subtyping indolent B-cell lymphomas. In fact, while immunocytoma carries both antigens, IRF4 is usually negative in the other types with the exception of chronic lymphocytic leukemia (B-CLL) in which prolymphocytes and paraimmunoblasts are usually positive. The BSAP antigen – as well as the *bcl6* product – is very helpful for recognizing the interfollicular component of follicular lymphoma (FL). CD10, *bcl6*, and *bcl2* antigens are useful in diagnosing both indolent and aggressive lymphomas. Among the former, CD10 and *bcl6* are positive in germinal center cells, thus diagnostic in FL and effective in differentiating this from B-cell lymphomas growing around residual germinal centers (mantle cell-, marginal zone cell-, small lymphocytic-lymphomas). The expression of the *bcl2* protein is widely present, although it bears t(14;18) only in FL: nonetheless, it is relevant in differentiating *bcl2*- follicular hyperplasias from FLs, the majority of which are positive. This triad of molecules shows different patterns in diffuse large B-cell lymphomas (DLBCL) and Burkitt's lymphoma. A CD10⁺/*bcl6*⁺/*bcl2*⁺ phenotype corresponds to germinal centers neoplasms, as probably does the CD10⁺/*bcl6*⁺/*bcl2*⁺ one. A negative CD10 is found in: a) immunoblastic lymphomas (which are also *bcl6*⁺, *bcl2*⁺, IRF4⁺, VS38C⁺, Clg⁺, and CD138⁺), b) lymphomas with anaplastic morphology (*bcl6*⁺, *bcl2*⁺, and CD30⁺), c) non-immunoblastic/non-anaplastic DLBCL, part of which are *bcl6*⁺ possibly because of *bcl6* gene rearrangement. As far as Burkitt's lymphoma is concerned, a CD10⁺/*bcl6*⁺/*bcl2*⁺ phenotype is observed, together with the overexpression of the *c-myc* oncogene product due to either t(8;14), t(2;8) or t(8;22). On the other side, the controversial variety termed Burkitt's-like lymphoma is *bcl6*⁺/*bcl2*⁺/CD10⁺. Concerning the ALK protein, one should remember that the definition of anaplastic large cell lymphoma (ALCL) has recently been modified due to the frequent detection of t(2;5)(p23;q35), that produces a hybrid gene (NPM/ALK), which encodes for a chimeric protein formed by the N-terminal region of nucleophosmin and the cytoplasmic domain of the tyrosine-kinase receptor ALK. Monoclonal antibodies have been developed against both the ALK and NPM proteins. In the presence of t(2;5), the former produce nuclear and cytoplasmic positivity, a cytoplasmic staining being observed only in cases carrying chromosomal aberrations other than t(2;5), but involving chromosome 2 at p23. Sixty percent of ALCLs (most of which are of the common and lympho-histiocytic type) are positive for the ALK protein. This finding is particularly relevant since it: 1) identifies ALCLs with a better prognosis, 2) allows the detection of minimal residual disease, and 3) represents a marker of malignancy, normal lymphocytes being negative.

DIAGNOSTIC IMPLICATIONS OF GENETIC LESIONS IN NON-HODGKIN'S LYMPHOMA

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Despite their common origin from mature lymphoid cells, non-Hodgkin's lymphomas (NHL) constitute an extremely heterogeneous group of diseases. First, NHL may derive from either the B-cell or the T-cell lineage. Second, within each lymphoid lineage, NHL may derive from cells at different stages of maturation. Finally, NHL differ in terms of pathogenetic pathways and patterns of molecular alterations. The Revised European-American Lymphoma (REAL) classification and the WHO proposal have recently included tumor genotype among classification criteria. Indeed, a large body of evidence accumulated during the last decade has shown that NHL tend to associate with specific genetic lesions representing the primary pathogenetic event. As a result, NHL subgroups previously thought to be nosologically distinct have been combined into a single entity, whereas NHL categories initially defined as a single uniform group have been dissected into specific subgroups. From a clinical standpoint, NHL genetic lesions represent molecular markers of disease serving three distinct purposes: 1) they assist and complement morphologic and immunohistochemical diagnosis; 2) they allow evaluation of minimal residual disease by highly specific and highly sensitive technologies; and 3) they provide prognostic indicators in some cases. Genetic lesions may serve as tools for NHL diagnosis because of the preferential association between a given genetic lesion and a distinct NHL category. Examples are the associations between lesions of BCL-1 and mantle cell lymphoma, BCL-2 and follicular lymphoma, BCL-6 and B-lineage diffuse large cell lymphoma, c-MYC and Burkitt's lymphoma, as well as HHV-8 infection and primary effusion lymphoma. The practical usefulness of genetic lesions for the correct classification of NHL may be readily demonstrated by specific examples. Thus, when considering non-follicular small cell NHL, the subgrouping of which is a traditionally difficult task for pathologists, BCL-1 alterations are considered the most specific clue for a diagnosis of mantle cell lymphoma. The possibility of monitoring the presence of neoplastic cells with a high sensitivity technique such as polymerase chain reaction (PCR) applied to studies of minimal residual disease represents a major tool for follow-up evaluation of human tumors. In the field of NHL, molecular analysis of minimal residual disease is now feasible for routine purposes in the case of BCL-1 and BCL-2 alterations. In these two groups of genetic lesions, the relative consistency of breakpoints at the nucleotide level allows a PCR based strategy by taking advantage of the JH consensus sequence on chromosome 14. Overall, the goal of

the application of molecular genetics to the diagnosis of NHL is the establishment of 100% associations between a given genetic lesion and a given lymphoma entity. Despite several major achievements, more work needs to be done toward this goal. In several NHL categories, the associated genetic lesions are conventionally detectable in the majority cases, although not in all. Several practical reasons may explain this discrepancy, including the failure of conventional laboratory assays to detect all the possible types of molecular lesions of a given cancer related gene, as well as the possibility of pathologic misdiagnosis among subtly different NHL types. At another level, however, it cannot be formally excluded that the genetic features of NHL are more heterogeneous than we presently assume.

EVALUATION OF RESPONSE (MINIMAL RESIDUAL DISEASE DETECTION)

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Current induction therapies for acute and chronic leukemias and the lymphomas have achieved significant complete remission rates. Despite this initial success, disease recurrence remains a major problem. Relapse from clinically undetectable residual malignant cells is the most likely mechanism of recurrence. Of crucial importance to the clinician is the accurate detection of residual malignant cells prior to clinical relapse. Standard approaches to evaluate this minimal residual disease (MRD) allow detection only when the malignant clone exceeds 1%. Patients in remission, however, may frequently have residual neoplastic cells that are far below this level. The study of minimal residual disease has been fuelled by the technological advent of the polymerase chain reaction (PCR) and basic developments identifying the genetic lesions involved in human malignancies. In the last decade several investigators have adapted PCR to detect tumor-specific DNA or RNA sequences. The application of this technique to the study of MRD has, thus far, been limited to tumors in which specific DNA or RNA sequence data are available. Specific PCR-amplifiable genetic lesions may be used (i.e. BCL2/JH rearrangement in follicular lymphomas, ecc....). PCR amplification of rearrangements of the complementary region III (CDRIII) of the immunoglobulin or T-cell receptors genes is also carried out in lymphoproliferative disorders lacking a detectable genetic defect. Both approaches are highly sensitive (able to detect 1 malignant cell in 10⁶ normal cells). Monitoring of residual disease in patients with malignant hematologic disorders is now recognized as an important diagnostic tool for assessment of the response to treatment and the individual risk of relapse. In a number of malignancies, however, employment of very sensitive techniques permitting the identification of tumor cells within a 10⁴ -fold or greater excess of normal cells revealed that the presence and persistence of residual disease at this level does not necessarily

imply inevitable relapse. Several studies demonstrate that surveillance of persisting neoplastic cells by a quantitative technique (i.e. real time quantitative PCR) can provide prognostically relevant information on the sensitivity of the malignant population to the treatment applied and the biological behavior of the residual tumor cell clone during and after therapy. Therefore, investigation of the level or the dynamics of residual disease by sensitive and quantitative approaches can provide a basis for clinical decision making and it is now considered an essential step in the clinical management of different types of leukemia and lymphomas.

NEW BIOLOGICAL PROGNOSTIC FACTORS IN HODGKIN'S DISEASE

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In Hodgkin's disease (HD), 15-35% of patients either progress during front-line therapy or relapse following the induction of complete remission (CR). Several attempts have been made to identify patients with inferior failure-free survival (FFS) and various prognostic models have been proposed along the years. However, none of them can confidently identify sizeable populations of patients with FFS far below 50%. More powerful prognostic models could perhaps be generated from the identification of new prognostic features derived from the combined analysis of clinical aspects and new biological markers related to the biological complexity of HD.

Biological factors potentially useful as prognostic markers in HD can be subdivided in three main groups: 1. Conventional hematologic and biochemical markers; 2. Cellular antigens (expressed by Hodgkin-Reed Sternberg [H-RS] and/or bystander cells) detected by immunohistologic techniques; 3. Serum/plasma molecules (including a number of cytokines and their soluble receptors and other soluble molecules). Conventional hematologic and biochemical markers include ESR, LDH, Hb level, WBC & lymphocyte counts, serum albumin, etc. Data on their prognostic role have been repeatedly reported. Overall, their predictive ability is variable and weak when evaluated in the context of all other known prognostic variables. Cellular antigens include: those related to H-RS cell proliferation (Ki-67, PCNA, MIB-1, cyclins/CDKs, RB, p16, p21, p27, others); those related to H-RS cell immunophenotype (CD15 and CD20); products of oncogenes and tumor-suppressor genes (p53, mdm2, bcl-2); EBV-related proteins (LMP-1). Some evidence suggest a prognostic role for some of them. Serum/plasma molecules investigated as potential prognostic markers include β_2 -M, TNF α , IL-6, IL-10, sIL-2R, sCD8, sTNFRs, sICAM, sVCAM, sCD27, sCD30 and others. With a few notable exceptions (sCD30 and IL-10), these markers have been investigated in small series and in non-

homogeneously treated patients. Although they often correlate with disease activity and AAS and outcome (survival, FFS, FFDP) in univariate analysis, only occasionally they maintain an independent prognostic significance in multivariate analysis. Based on the information available, time appears mature for the search for a new prognostic model derived from a large multi-institutional study investigating the impact of biological markers with potential prognostic value. For this purpose, the databases of the MDACC (Houston), Mayo Clinic (Rochester), Istituto Tumori (Milan), and Hematology Depts. of Verona and Athens have already merged and include 1767 patients with serum and tissue available. This database has allowed identification of the prognostic value of IL-10, sCD30, β_2 -M, LDH, serum albumin, and the expression of bcl-2, CD20, and LMP-1 by H-RS cells. These individual prognostic factors will be entered into multivariate analyses to identify those which are statistically independent. It is thus hoped that a sizeable fraction of patients with PFS less than 50% will be identified, who will possibly benefit from experimental therapy.

NEW INSIGHTS INTO THE LYMPHOPROLIFERATIVE DISEASE OF GRANULAR LYMPHOCYTES

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An increased number of granular lymphocytes (GL) has been reported in various clinical conditions and is currently interpreted as a reactive process to an underlying antigenic stimulation. In recent years, a disease characterized by a definite increase of granular lymphocytes has been identified and recognized as lymphoproliferative disease of GL (LDGL). Among the lymphoproliferative diseases, LDGL accounts for approximately 5% of chronic disorders. The criteria for diagnosis of LDGL have been recently updated, by demonstrating that the previous adopted criteria, i.e. an absolute GL number greater than 2,000/ μ L, is not essential for the diagnosis, provided that the expansion of a discrete GL population can be demonstrated using immunologic or molecular methods. These include the demonstration of a dominant TCR $\nu\beta$ populations using specific monoclonal antibodies by flow cytometry or the skewing of TCR $\nu\beta$ repertoire demonstrated by PCR or the detection of discrete cell population recognized by the expression of the p58 related antigens CD158a and CD158b on the cell surface. Immunologic classification of this disease distinguishes a CD3⁺ form, more common, and a CD3⁻ variant; this latter accounting for nearly 15% of LDGL cases. CD3⁺ LDGL is symptomatic in approximately 50% of cases, neutropenia, infections and anemia being the most frequent findings. Clonality of the T-cell receptor is usually documented in these patients. Cytokines such as IL-2, IL-12 and IL-15 have been claimed to play a role in this disorder. Symptomatic patients may benefit from combination therapy with low dose methotrexate and steroids. CD3⁻ LDGL are usually associated with viral infection of

GL: in particular, Epstein-Barr and human T-lymphotropic virus I/II have been claimed to have role. Neutropenia is usually less pronounced than in CD3⁺ LDGL patients. Clonality has rarely been demonstrated; however, when present, it correlates to an aggressive clinical course. A spontaneous regression of lymphocytosis has been reported in both CD3⁺ and CD3⁻ patients. Care must be taken in distinguishing patients with LDGL from those characterized by a reactive lymphocytosis. In fact, an increase in GL can be seen in many clinical conditions, including viral infections (EBV, HBV, HCV, HIV, CMV), idiopathic thrombocytopenic purpura, skin disorder and hemophagocytosis: in these conditions clonality is not detectable and the lymphocytosis is self limiting.

According with the above quoted features, LDGL is a well recognized disorder which encompasses a large spectrum of conditions, ranging from mild asymptomatic lymphocytosis to aggressive, usually fatal, disorders. Diagnosis of this disease is related to the demonstration that a discrete subset of GL is chronically expanded. Therapy should be delayed in asymptomatic patients; however, when needed, the combination of methotrexate or cyclophosphamide and steroids represents the most promising approach.

4th session
NON-HODGKIN'S LYMPHOMAS
(S. Tura, S. Horning)

THERAPY FOR DIFFUSE AGGRESSIVE LYMPHOMA

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The CHOP regimen for diffuse large cell lymphoma was introduced in 1976. Subsequently a number of multi-agent chemotherapy regimens featuring new drugs, more continuous treatment and dose intensification have been tested. Despite early enthusiasm, the results of mature phase III trials have been disappointing. Acceptance of an international prognostic factors index affords study of like patients. In addition to clinical factors, a number of biological prognostic factors have been identified, including bcl-2, p53, inhibitors of cyclin-dependent kinases and adhesion molecules. These may provide clues for future treatments as well as providing prognostic information. Recently, molecular profiling in a group of 37 diffuse large cell lymphomas demonstrated prognostic significance according to gene expression patterns beyond the clinical parameters in the International Index. A global effort to expand this database is underway. Multiple randomized trials in Europe have addressed the ability of myeloablative therapy to overcome adverse prognosis in diffuse aggressive lymphoma. These studies differ in design and prospective versus retrospective assignment of prognostic features. Study variables and interpretation of results, which are varied, will be provided. Rituximab combined with

CHOP has been shown to be well tolerated and randomized phase III studies are in progress. It will be very important to evaluate biological parameters during the course of these clinical trials.

AGGRESSIVE NON-HODGKIN'S LYMPHOMAS: IS AGE STILL A LIMIT TO TREATMENT?

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Lymphomas belong to the class of malignant disease with the fastest increasing incidence. High-grade non-Hodgkin's lymphomas (HG-NHL) display a peak of incidence in the age group above 65 years old. In the last few years, age has been recognized as a major risk factor for overall survival of HG-NHL patients, and numerous attempts have been made to analyze the contribution of factors to the age-related worsening of prognosis in this disease. Several authors have concluded that age is an important prognostic factor in the treatment of these patients. Following this analysis, effective chemotherapy regimens have recently been specifically designed and tested on elderly patients with HG-NHL. To minimize the toxicity associated with outpatient chemotherapy regimens, we designed an 8-week pilot regimen, VNCOP-B, for treating HG-NHL using moderate doses of chemotherapy at frequent dosing intervals, and obtained a good remission rate. In 29 patients we observed an overall response rate of 93%, with a complete response rate of 76%. Subsequently, we commenced a multicenter randomized trial including granulocyte colony-stimulating factor (G-CSF) as a further component of treatment to determine whether toxicity could be further reduced without sacrificing efficacy. The VNCOP-B program is similar to a MACOP-B-like regimen but with several distinctive features: in particular, treatment is completed in 8 weeks and includes mitoxantrone and etoposide instead of doxorubicin and methotrexate, respectively. We selected these replacements for the purpose of reducing the incidence of cardiac side effects and mucositis. Drug doses, including prednisone, were slowly reduced; all treatment was given on an outpatient basis. The G-CSF administration was 5 µg/kg/day subcutaneously throughout the treatment, starting on day 3 of every week for 5 consecutive days. Of the 158 patients enrolled for the trial, 149 patients were evaluable: 77 received VNCOP-B plus G-CSF and 72 received VNCOP-B alone. The overall response rate was 81.5%, with complete response in 59%: 60% in the VNCOP-B plus G-CSF group, and 58% in the VNCOP-B group. At 48 months (median 38 months), 62% of all complete responders were alive without disease in the G-CSF group and 60% in the control group. Neutropenia occurred in 18 out of 77 (23%) of the G-CSF treated patients and in 40 out of 72 (55.5%) of the controls ($p=0.00005$). Clinically relevant infections occurred in 4 out of 77 (5%) of the G-CSF group and in 15 out of 72 (21%) of the controls ($p=0.004$). Our data show that VNCOP-B is a feasi-

ble and effective regimen as regards the complete response rate and relapse-free survival rate in elderly patients with advanced HG-NHL; the use of G-CSF reduces infection and neutropenia rates.

TREATMENT OF AGGRESSIVE NON-HODGKIN'S LYMPHOMAS ACCORDING TO PROGNOSTIC FACTORS. LESSONS FROM THE GISL LA03 STUDY

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Age, stage, extranodal involvement, performance status, and serum lactate dehydrogenase level represent important factors affecting the course of aggressive non-Hodgkin's lymphoma (NHL). Using these factors the International Prognostic Index (IPI) was developed, allowing the identification of four risk groups. In 1993 the Gruppo Italiano per lo Studio dei Linfomi (GISL) started a randomized 2x2 factorial study (comparing a flexible versus fixed dosing schedule of two different anthracycline-containing ProMACE-CytaBOM regimens) in which the duration of the treatment was modulated according to the IPI. After four courses of ProMACE-CytaBOM, responding patients with low or low intermediate IPI (low risk) were planned to receive 2 additional courses whereas those with intermediate-high or high IPI (high risk) were planned to receive 4 additional courses of chemotherapy. Between July 1993 and June 1997, 356 patients with advanced aggressive NHL were registered for the study. After randomization 15 patients were considered ineligible (9) or withdrew (6) from the study before the first assessment of response. The remaining 341 patients were included in the analysis. No statistical differences between study arms were observed as regards the baseline characteristics. After four courses of therapy, 53% of patients in the low risk and 38% in the high risk group achieved a complete remission (CR) ($p = 0.001$). At the end of induction therapy 321 patients were assessable for response. The rate of CR was 75% and 52% for patients at low and high risk respectively ($p = 0.0001$). Interestingly, the conversion from partial remission (PR) to CR with further therapy (2 or 4 courses depending on the risk group) was similar (31% and 35%) in both groups. After a median follow-up of 33 months (44 months for patients alive), 127 patients (37%) had died, 22 had been lost to follow-up (6%) and 198 were alive. The 3-year and 5-year estimated survival rates were 75% and 67% for the low risk and 47% and 41% for the high risk group, respectively ($p < 0.0001$).

The preliminary results of our study suggest that 6 courses of ProMACE-CytaBOM produced a promising success rate in patients with advanced aggressive NHL and a low or low intermediate IPI score, whereas the same regimen was less effective in patients with an intermediate or high IPI score, even if 2 additional courses were delivered. In this latter group of patients most failures were observed during the first 4 courses of therapy, suggesting that innovative approaches should be considered up-front.

CONVENTIONAL TREATMENT OF FOLLICULAR LYMPHOMAS

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Follicular lymphomas (FL) account for approximately 25% of NHLs in Europe. Their treatment raises several questions for the following reasons: (i) their course is slow with a median survival around 8-10 years and the initial treatment must have limited toxicity; (ii) they usually respond to initial treatment but relapse is the rule and the response to subsequent treatments is less complete and durable; (iii) there is a risk of histologic transformation (7-10% per year) and the prognosis after transformation is very poor (median survival, 1 year). The choice of initial treatment must rely on prognostic criteria. Several retrospective studies have revealed prognostic factors but these require confirmation in other series. An international project for proposing a prognostic index is on-going and the characteristics of more than 5000 patients have been collected. Radiation therapy (40 Gy in the involved field) remains the standard treatment of Ann Arbor stage I-II follicular lymphomas. Combined therapy may be proposed to patients with bulky or stage II disease. There is no consensus on the treatment of patients with disseminated disease. For those with a low-tumor burden, watchful waiting may be proposed since there is no demonstration that chemotherapy improves progression-free and overall survival rates in these patients. A recent study of rituximab treatment may modify this strategy. For patients with a high tumor burden, chemotherapy is required to alleviate symptoms and induce tumor regression. There is no consensus on the type of chemotherapy to be used. In several studies, the Groupe d'Etude des Lymphomes Folliculaires (GELF, France, Belgium) has shown that a combination of an adriamycin-containing regimen and interferon- α was clearly superior, in terms of progression-free and overall survival rates, to the same regimen given alone or to single treatment with fludarabine. This association has become the standard for several groups but the potential toxicity of interferon alpha and its consequences on quality of life have raised concerns in many physicians. Several phase II studies have suggested a favorable role of intensive therapy with autologous stem cell transplantation. A definitive answer will soon be given by randomized studies being conducted by the EORTC, the GELF and other groups in France and Germany.

HIV-RELATED NON-HODGKIN'S LYMPHOMA: GENERAL CHARACTERISTICS AND RESULTS OF TREATMENT ADAPTED TO PROGNOSTIC FACTORS

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In recent times, the field of HIV-related non-Hodgkin's lymphoma (NHL-HIV) has been influenced by several novel discoveries. First, biological studies have

led to the identification of new clinico-pathologic types of AIDS-related lymphomas (primary effusion lymphomas, plasmablastic lymphomas of the oral cavity) which were previously unrecognized as independent diseases. Second, the epidemiological differences between NHL-HIV and Kaposi's sarcoma (KS) have been reinforced by the striking reduction of KS in HAART-treated patients, whereas the frequency of systemic NHL-HIV has remained substantially unvaried despite more effective antiretroviral therapy. Typically, patients with systemic NHL-HIV present with widespread disease and extranodal involvement at diagnosis, the most common sites being the central nervous system, gastrointestinal tract, bone marrow and liver. Several prognostic factors predictive of survival have been identified, including immune function, a prior AIDS diagnosis, Karnofsky performance status, age, lactate dehydrogenase level and response to therapy. The treatment of systemic AIDS-NHL is a great challenge. Traditionally, in fact, low dose chemotherapy has been considered standard treatment for all patients. However, although the response rate is high, the median survival of these patients is approximately 6-10 months and only 10-20% survive free of disease for longer than 2 years. Since the advent of HAART, prolongation in life expectancy and reduction of opportunistic infections of HIV-infected patients have questioned the value of a conservative approach with low dose chemotherapy.

In May 1993 the European Intergroup Study NHL-HIV started a randomized study with a stratification of lymphoma patients into three groups according to the presence or not of adverse prognostic factors, prior AIDS, $CD4^+ < 100/mm^3$, performance status > 1 . In the low risk group (i.e. absence of any of the three prognostic factors) patients were randomized between the intensive regimen ACVB or CHOP with G-CSF support. One hundred and ninety-two patients have been included, 98 in the ACVB and 94 in the CHOP arm. Forty-three per cent had diffuse large cell (DLCL) lymphoma. Sixty-one per cent had an international prognostic index (IPI) score ≥ 2 . Median $CD4$ count was $229/\mu L$. Complete response (CR) was achieved in 65 % and 56 % for respectively ACVB and CHOP ($p = 0.2$). Survival and event-free survival (EFS) were similar. Fifty per cent of deaths from AIDS while patient were still in CR were observed during the follow-up. In the intermediate risk group (with the presence of only one prognostic factor) patients were randomized between CHOP or CHOP reduced to 50 %. Of the 141 patients, 75 were in the CHOP arm and 66 in the 50 % reduced CHOP. Forty-nine per cent had DLCL. Sixty-three had an IPI score ≥ 2 . Median $CD4$ count was $61/\mu L$. The 59 % CR rate in the CHOP arm was superior to the 35 % in the reduced CHOP arm ($p = 0.005$). Overall survival rate was 35% in the CHOP arm vs 28% in the reduced CHOP one ($p = 0.09$) and EFS, 33% vs 25% ($p = 0.04$), respectively. Twenty per cent of patients died while in CR. CHOP can be considered the standard treatment for patients with NHL-HIV.

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GERIATRIC ASSESSMENT IN THE MANAGEMENT OF ELDERLY PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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The poorer outcome of older patients with aggressive non-Hodgkin's lymphoma (NHL) may be related not only to a poorer initial response to all regimens, but probably to an increased treatment-related toxicity. Some age-associated conditions are probably responsible for the increased risk of toxicity and the lesser tolerance of intensive combination chemotherapy. The medical oncologist and the hematologist managing older persons with non-Hodgkin's lymphoma (NHL) are faced with the following questions: will this patient die of NHL or unrelated conditions? Can the patient tolerate cytotoxic chemotherapy in full doses? The answer to these questions may be provided by a multidimensional assessment of the older person that accounts for individual variations in life expectancy, health and ability to withstand stress. A multidisciplinary assessment of this type has already been successful in several areas of geriatric medicine, including preservation of independence, prevention of hospital admission and of falls and management of acute conditions such as delirium. Several aspects of geriatric care should be assessed. Functional assessment in terms of activities of daily living (IADL) is important: the short-term (2 year) mortality increases with the degree of dependence. Functional dependency may amenable to rehabilitation, dependence in ADLs is a criteria of frailty, and dependence in IADLs may suggest decreased tolerance to chemotherapy. The prevalence of comorbid conditions increases with age and may lead to increased mortality. Careful assessment of the health status may also reveal unsuspected and undetected diseases that compromise the management of cancer, such as heart failure, renal dysfunction, anemia or diabetes.

Both dementia and depression are associated with increased mortality. The mini-mental status (MMS) allows dementia to be graded as mild, moderate or severe, and the geriatric depression scale (GDS) is a useful screening tool for depression. Both conditions may also reduce the understanding of, and the motivation for, cancer treatment. Management of depression may improve the cognitive status and the quality of life of the patient and foster treatment acceptance. Recognition of a frail person is of obvious importance. Frailty is defined by one of the following criteria: aged 80 years and over; presence of one or more geriatric syndromes, three or more comorbid conditions; one or more ADL dependence. The frail person has exhausted almost all functional reserves and is generally a candidate for single agent chemotherapy only or palliative radiotherapy.

5th session
HIGH DOSE CHEMOTHERAPY
 (A.H. Goldstone, V. Rizzoli)

HIGH DOSE THERAPY IN FOLLICULAR LYMPHOMA

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High dose therapy (HDT) has been widely adopted as a potentially curative modality for patients with recurrent aggressive non-Hodgkin's lymphoma. However, its use in follicular lymphoma (FL) has been viewed with caution and remains experimental. To overcome the risk of reinfusing lymphoma cells present in the bone marrow collection, the use of anti-B cell antibodies and complement lysis at St Bartholomew's Hospital (SBH) followed the original observation that this was feasible at the Dana Farber Cancer Institute (DFCI), Boston. Recently, SBH has reported long term clinical (median follow-up, 6 years) and molecular follow-up results for patients receiving HDT (Cyclo/TBI) and autologous bone marrow support as consolidation of second or subsequent remission. Although the results confirm previous reports indicating that HDT improves freedom-from-recurrence (FFR) ($p < 0.001$), in terms of improved survival the validity of this approach has yet to be demonstrated ($p = \text{NS}$). Although such an advantage could emerge later, it might be precluded by the unexpected high incidence of therapy-related deaths due to s-MDS (12% incidence at 6 years in the SBH study). Recurrence remains the major cause of treatment failure, observed as late as 8 years after HDT, reflecting the relatively long clinical course of the illness. The majority of patients present with recurrence at previous sites of disease, suggesting that this is due to endogenous lymphoma resistant to myeloablative doses of therapy rather than to reinfusion of lymphoma cells present in the bone marrow collection. The survival patterns after recurrence for these patients suggest that HDT does not compromise outcome in patients in whom it fails, reflecting the survival pattern of the disease when treated conventionally. In the SBH study, the increase from one to four anti-B cell antibodies did not improve the efficacy of purging (25% and 31%, respectively, $p = 0.73$). Similarly, there was no statistical advantage in receiving *clean* as opposed to *contaminated* bone marrow to support HDT, as suggested by other groups. More important, this study has confirmed that recurrence and death is most unlikely in patients who have *undetectable* numbers of cells bearing the Bcl-2/IgH rearrangement in the bone marrow at follow-up (HR, 0.13; $p < 0.001$ and 0.25; $p = .02$, respectively). This is entirely in keeping with the results of identical therapy at the DFCI, is supported by results obtained with conventional intensive therapy as given at the

M.D Anderson Cancer Center, and lends support to the concept that molecular remission is a goal worth achieving. In the absence of a less toxic alternative, it seems appropriate to continue to investigate HDT as part of treatment for FL, even though in its present form, it is not curative for at least half of those treated. However, it is imperative to improve the efficacy of the treatment and to try to reduce the incidence of s-MDS. Whether the use of peripheral blood progenitor cells, variously purged *ex vivo*, will reduce the recurrence rate remains to be proven. The concept of using anti-CD20 therapy before the collection of stem cells is clearly attractive. There is strong evidence that the blood and bone marrow compartment are at least transiently cleared of cells containing the Bcl-2/IgH rearrangement. One can argue that HDT should be followed by additional therapy (e.g., interferon), as is being investigated, or, alternatively, by anti-CD20 therapy. Given that most recurrences after HDT occur in the first 2 years, such additional therapy should probably be given early, after HDT. The use of quantitative, real time PCR may shed light on the optimal time point for such therapeutic interventions. Recurrence and the unexpected high incidence of s-MDS following HDT and autologous stem cell support has renewed the interest in the role of allogeneic transplantation in FL. Although not applicable to the majority of patients, non-myeloablative or mixed chimeric allotransplantation is currently being investigated. It may take time to demonstrate whether any of these approaches are curative, or whether a new algorithm needs to be constructed.

DETECTION OF MINIMAL RESIDUAL DISEASE IN HEMATOLOGIC MALIGNANCIES AFTER HIGH DOSE CHEMOTHERAPY

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High dose therapies (HDT) with stem cell rescue have enabled the majority of patients (pts) with high-risk-hematologic malignancies to obtain long disease free survival, or cures. However a percent of these patients relapse, usually with the same malignant clone found at diagnosis, demonstrating the persistence of low number of malignant clonogenic cells (minimal residual disease: MRD). The detection of MRD offers the opportunity of monitoring the evolution of disease and for early intervention before relapse, although in some malignancies residual tumor cells may persist without any evidence of subsequent relapse. The identification of MRD requires the presence of a malignant marker specific for neoplastic cells and techniques able to recognize them. The methods usually used for the routine evaluation of remission are morphology, immunophenotyping, cell culture assay, cytogenetics, but the most sensitive technique for detection of MRD is the polymerase chain reaction (PCR) which exponentially amplifies specific DNA sequences which are unique to the malignant clone. Efforts have been made to establish whether a persis-

tence of MRD detectable with PCR analysis after HTD is predictive of relapse, but the results are heterogeneous. In chronic myeloid leukemia large studies showed a clear association between detectable PCR 6-12 months post allogeneic transplant and relapse. In chronic lymphoid leukemia data on PCR analysis suggest that patients who were PCR negative and become PCR positive after HDT tend to relapse. In acute leukemias, MRD detection in the first 100 days after stem cell transplantation (SCT) is associated with a poor prognosis compared to prognosis in PCR negative patients. In acute lymphoblastic leukemia patients with p190 bcr/abl have a relapse rate higher than patients expressing the p210 bcr/abl transcript. In acute myeloid leukemia (AML) the significance of a qualitative assay of cytogenetic alterations is debatable: the MRD in t(8;21) AML may persist for long time after SCT without evidence of relapse but, in t(15;17) AML, the MRD detected by PCR is highly predictive of relapse. In lymphoma and other malignancies the data are controversial due to histologic types, heterogeneity of conventional therapies, conditioning regimens, the presence of t(14;18); in high grade lymphoma it seems that other methods such as FISH could provide a better understanding of the biology of these diseases. Finally the PCR assay could be used in autologous SCT to assess the efficacy of *ex vivo* purging before reinfusion. In conclusion there is not agreement on the utility of PCR for prediction of relapse after HDT in all hematological malignancies; however the use of this technique, possibly associated with other methods such as FISH, seems to be the best way to evaluate the probability of relapse in some cases and is of great interest for biological and clinical studies of *ex vivo* purging for autologous transplant.

HIGH-DOSE SEQUENTIAL CHEMOTHERAPY: A FEASIBLE AND EFFECTIVE REGIMEN FOR HIGH-RISK NON-HODGKIN'S LYMPHOMA

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Autologous transplantation is frequently used as frontline or salvage treatment in non-Hodgkin's lymphoma (NHL). Several studies suggest that myeloablative regimens are most effective when delivered after maximal cytoreduction. Based on this concept, the high-dose sequential (HDS) chemotherapy regimen was designed, including an initial sequential high-dose phase, followed by circulating progenitor cell autograft. In the first experience, the original HDS regimen was found superior to MACOP-B as frontline treatment in a subset of poor prognosis diffuse large B-cell lymphoma, in terms of complete remission (CR) achievement and event-free survival (EFS). Thereafter, *second generation* HDS regimens were developed to achieve two main goals: a) to increase further the effectiveness in poor prognosis high-grade NHL (Ara-C-HDS); b) to develop a suitable schedule for patients with indolent lymphomas. So far, 122 patients (62 high grade, 60 indolent) have been treat-

ed at disease onset; in addition, 48 patients with relapsed/refractory disease were managed with such an HDS approach. Overall, there have been 8 toxic deaths (transplant-related mortality=5%); secondary neoplasias were recorded in 5 patients. (3%). Results according to the two main programs were as follows: (a) high-grade lymphomas: the long-term outcome shows a 3-year EFS of 72%, confirming the results of the randomized study in a wider and extremely high-risk patient population (B and T-cell NHL, a.a. IPI score > 2-3); (b) indolent lymphomas (28 follicular, 32 non-follicular): results were highly satisfactory with a CR rate of 74% and a median overall survival not yet reached at a median follow-up of 5 years. In addition, a persistent clinical and molecular remission was achieved in 50% of follicular lymphoma patients; in contrast, molecular remissions were rare in non-follicular subtypes. Based on these results, a multicenter trial was performed with HDS specifically suited to indolent lymphoma in high-risk follicular lymphoma. Clinical and molecular results of this latter study were superimposable to those of the pilot study. The most recent progress in the treatment of NHL was the introduction of monoclonal antibodies and in particular rituximab. In the newly developed *third-generation* HDS regimens, rituximab has been associated with HDS chemotherapy (R-HDS) in order to obtain an *ex vivo* purging effect before stem cell collection. In a recent trial, R-HDS has allowed the collection of PCR-negative harvests in all 11 mantle-cell lymphoma patients so far treated. Based on these promising results we are now using R-HDS in many different therapeutic settings. In particular a randomized multicenter trial comparing R-HDS vs CHOP + rituximab has been recently launched in 40 Italian centers. In conclusion, our experience with HDS chemotherapy shows that this treatment is feasible and effective and can be successfully combined with novel therapeutic tools now entering the clinical arena.

ALLOGRAFTING FOR HIGH-RISK PATIENTS: REDUCED INTENSITY CHEMOTHERAPY WITH THIOTEPA, FLUDARABINE, AND CYCLOPHOSPHAMIDE ALLOWS A STABLE ENGRAFTMENT WITH LOW TOXICITY

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Allogeneic transplantation represents not only a way to restore hematopoiesis after chemoradiotherapy, but also a form of adoptive immunotherapy. Allografting efficacy is, however, frequently hampered by its toxicity. This is particularly true when candidates are older than 45 years, heavily pretreated, or have other comorbid conditions. In order to decrease treatment-related morbidity/mortality (TRM) and to enhance the graft-versus-tumor effect, we have developed a strategy in which reduced intensity chemotherapy (thiotepa 15 to 5 mg/kg, fludarabine 60 mg/m², cyclophosphamide 60 mg/kg) is associated with low-dose GVHD prophylaxis (cyclosporinA[CA] 1 mg/kg,

methotrexate 10 mg/m² d+1, 8 mg/m² d+3, d+6), and programmed reinfusions of engineered (TK+) lymphocytes (CyA tapering at d+90: reinfusion of 1x10⁷/kg cells for patients with molecular disease, or 1x10⁸/kg cells for those with clinical disease). We have conducted a pilot study, enrolling 21 patients with a median age of 51 years (range 33-65). Nine had non-Hodgkin's disease, 1 Hodgkin's disease, 3 acute lymphoblastic leukemia, 3 breast cancer, 4 refractory anemia with excess blasts in transformation, 1 acute myeloid leukemia and 2 renal carcinoma. Six had early stage disease, 15 advanced stage. Patients were considered at high-risk of TRM because they were old, heavily pretreated [8 relapsed after ASCT], and/or had concomitant organ dysfunction. Nine patients received bone marrow and 12 peripheral blood progenitor cell grafts. All patients engrafted; median time to achieve 500 neutrophils was 13 days, median time to 20,000 platelets was 19 days; chimerism at day +30 and +90 was full donor in all patients achieving remission; median follow-up is 221 days (range 36 – 550). Acute GVHD >2 was recorded in 1 patient, and 3 patients experienced WHO organ toxicity >2. Two patients died of disease progression and 1 of TRM. Thirteen patients had a disease response, including 2 molecular remissions, but 5 of them already relapsed. Five patients received donor lymphocytes: 1 had a minimal and 1 a complete response.

NEW ROLES FOR ALLOGENEIC TRANSPLANTATION IN LYMPHOMA

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Many factors have contributed to the return of allogeneic transplantation as a viable option in lymphoma. Amongst them are the reduction of transplant toxicity for conventional sibling transplants, the reduction of toxicity for mini transplants from with siblings or matched unrelated donors, and the actual availability of matched unrelated donors which has increased significantly as the numbers of volunteer donors or panels increase worldwide.

In many situations, allogeneic transplant for lymphoma has therefore become a viable option despite the fact that there is little concrete evidence of a graft-versus-lymphoma effect, particularly in Hodgkin's disease. Amongst those considered for allogeneic transplant now are patients who have a matched sibling and have previously failed an autograft, patients who failed to mobilize peripheral blood stem cells, and patients who, although in good status and with minimal disease, have modest minimal disease in the marrow. In addition to this, it is quite clear that some groups of lymphoma patients do particularly badly with an autologous transplant and they would include patients with extensive Hodgkin's disease failing initial chemotherapy very early in the first six to twelve months and any patient with low-grade lymphoma who would previously have been a candidate for autograft, since the outcome results for autolo-

gous transplantation in low-grade lymphoma show no plateau of event-free survival.

In fact, such is the modesty of the toxicity for a mini allograft that any lymphoma patient beyond second relapse with controlled disease who has a sibling match is now a candidate for a mini allograft, in some cases ahead of an autograft, although it must be emphasized that the progression-related outcome of such transplants still remains completely unclear in the medium term.

Main Symposium RITUXIMAB (T. Barbui)

MOLECULAR ERADICATION OF BCL2/IgH CHIMERIC GENE IN FOLLICULAR NON-HODGKIN'S LYMPHOMA PATIENTS AFTER SEQUENTIAL THERAPY WITH CHOP AND RITUXIMAB

Rambaldi A, Lazzari M, Manzoni C, Alterini R, Baccarani M, Barbui T, Bernasconi C, Gattei V, Lauria F, Lazzarino M, Mandelli F, Marotta G, Morra E, Pulsoni A, Tura S, Zagonel V, Zaja F, Zinzani PL, Gamba E, Dastoli G, Reato G, Foa R

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This multicenter study was designed to assess the effect of sequential administration of CHOP (6 cycles) and the humanized anti-CD20 monoclonal antibody rituximab in untreated follicular non-Hodgkin's lymphoma patients (stage II or more) with a molecularly proven t(14;18). The main endpoint of this study was to evaluate minimal residual disease by sequential PCR monitoring of t(14;18) performed 12, 28 and 44 weeks after baseline on bone marrow (BM) and peripheral blood (PB) lymphocytes. Patients who at the end of the last cycle of CHOP proved PCR positive on two determinations (baseline), were eligible for anti-CD20 administration (375 mg/m²/weekly for 4 weeks). Patients were recruited in 10 centers, while the molecular analyses were centralized in 2 laboratories (Bergamo, Turin). After 6 cycles of CHOP chemotherapy, 44 of the 122 evaluable patients (36%) proved PCR negative (on two determinations) both on BM and PB lymphocytes, and 78 PCR positive patients were assigned to receive rituximab. At the first follow-up control after rituximab therapy (12 weeks after baseline), 30 of the 58 patients (52%) so far analyzed showed a complete molecular response (PCR negativity in the BM and PB). A further increase of the molecular response was documented at the second follow-up (28 weeks from baseline), when 36 of the 47 patients analyzed (77%) were found to be PCR negative, thus indicating an apparent progressive clearance of the neoplastic clone and suggesting a prolonged *in vivo* effect of the anti-CD20 antibody. After 44 weeks, the percentage of PCR negative patients declined since 13 of the 26 patients analyzed (50%) remained PCR negative in both the BM and PB. Taken together, this interim analysis indicates that in a significant proportion of

t(14;18) positive follicular lymphomas, CHOP alone may lead to PCR negativity and that an effective *in vivo* purging of residual disease after CHOP can be obtained by 4 weekly infusions of rituximab. Whether obtaining PCR negativity has clinical-prognostic implications remains to be determined through a sufficiently prolonged follow-up.

MOLECULAR RESPONSES WITH RITUXIMAB IN NEWLY TREATED NON-HODGKIN'S LYMPHOMA WITH A LOW TUMOR BURDEN

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Treatment of follicular lymphoma patients with a low tumor burden can be delayed until disease progression, but this approach remains unsatisfactory since all patients will ultimately relapse. Rituximab, a chimeric monoclonal antibody which binds specifically to the CD20 antigen, induced an objective response in about 50 % of patients with relapsed low-grade or follicular B cell non-Hodgkin's lymphoma (NHL) with few side effects and toxicities. Therefore, we aim to evaluate the clinical and molecular activity of rituximab as single first-line therapy of patients with follicular non-Hodgkin's lymphoma. Fifty patients with follicular CD20 positive NHL with a low tumor burden were included in this prospective phase II trial and forty-nine were analyzed for clinical and molecular (bcl2-J_H gene rearrangement) responses at serial time points. The overall clinical response rate at 3 months was 73% (36/49) with 13 CR/CRu, 23 PR, 10 stable disease and 3 progression. Within the study population, PCR data on bcl2-J_H rearrangement was obtained from 32 patients. At day 50, 17/30 patients (57%) were negative for bcl2-J_H rearrangement in peripheral blood (PB) and 9/29 (31 %) negative in the bone marrow (BM). A significant ($p < .0001$) association was observed between molecular and clinical responses. After 12 months, 16/26 patients (62 %) were PCR negative in PB (12/16 also negative in BM). The kinetics and duration of the molecular response were assessed by comparing the results obtained at day 50 and after 12 months in patients from whom serial samples had been obtained and analyzed. Interestingly, all patients whose PB and BM were PCR negative at day 50 remained PCR negative in both sites at 12 months and 6 others patients became PCR negative in both sites. Interestingly, only 1 out of 17 patients who were PCR negative in PB at day 50 had disease progression during the first year, as compared to 8 of the 13 patients with PCR positive results (log-rank $p < .005$). Our results indicate that early molecular responses can be sustained for up to 12 months and that this response is strongly correlated with progression free-survival. Rituximab has a high clinical activity and a low toxicity and induces a high complete molecular response rate in previously untreated patients with follicular NHL with low tumor burden. Patients with a molecular response after rituximab may have a long-term remission and rituximab might constitute a curative approach to follicular NHL.

PILOT TRIAL OF RITUXIMAB AND CHEMOTHERAPY WITH INFUSED CYCLOPHOSPHAMIDE, DOXORUBICIN, AND ETOPOSIDE IN HIV-ASSOCIATED NON-HODGKIN'S LYMPHOMA

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Background. The objective of this trial was to determine the feasibility of combining rituximab with infused cyclophosphamide, doxorubicin and etoposide (CDE) (*Proc ASCO 18; 12a, 19991 abstr 41*). The rationale for adding rituximab includes its activity in refractory lymphoma, its non-overlapping toxicity and differing mechanism of action.

Methods. Twelve patients with HIV-associated B-cell non-Hodgkin's lymphoma (NHL) received infusional CDE [cyclophosphamide (200 mg/m²/day), doxorubicin (12.5 mg/m²/day), and etoposide (60 mg/m²/day) given by continuous intravenous infusion for 4 days (96 hours)] every 4 weeks for up to 8 cycles plus rituximab (375 mg/m²) by one of two schedules: (1) prior to each cycle of CDE (n = 7), (2) on day -8 and day -1 prior to cycle 1, just prior to cycles 3 and 5, then on days 28 and 35 after the last cycle (n = 5). **Results.** Patient characteristics: median CD4 138/μL (range 7-418); stage IV (n = 7 [58%]); intermediate (n = 6 [50%]) or high-grade (n = 6 [50%]) histology, intermediate-high or high-risk by the age-adjusted International Prognostic Index (n = 6 [50%]). The median number of rituximab doses given was 5 (range 2-6) and of CDE was 5 (range 3-6). There were 6 grade 3-4 infections that occurred in 4 patients (33%). The incidence of grade 3-4 toxicity for CDE rituximab compared with historical data for CDE alone (*Proc ASCO 18; 12a, 1999, abstr 41*) was comparable for infection (33% vs. 27%), neutropenia (75% vs. 85%), thrombocytopenia (42% vs. 75%), and mucositis (25% vs. 12%). Nine of 12 patients (75%) have had a complete response, and none has relapsed after a median of 4 months (range 1-13 months).

Conclusions. These findings suggest that the addition of rituximab to infused CDE does not substantially increase the risk of infection in patients with HIV-associated NHL, and that the combination is effective and merits further study.

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RITUXIMAB IN AUTOIMMUNE DISEASES

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Several diseases or syndromes are associated with polyclonal or monoclonal immunoglobulins that have abnormal physical properties and in many cases react or cross react with self antigens carried by erythrocytes, platelets and other normal cells. Since immunoglobulins are produced by plasmacells that originate from B-lymphocytes and CD20 is a B-lym-

phocyte lineage marker, the use of an anti-CD20 monoclonal antibody can help to control the secretion of the abnormal immunoglobulins. For this purpose, we are currently testing the therapeutic effect of the human-mouse chimeric monoclonal antibody rituximab (Mabthera, Roche) in cases of immune or autoimmune disease. Rituximab is given at a fixed dose of 500 mg, corresponding to 240 to 330 mg/m², weekly, for a total of four doses. All the cases were resistant to full dose and long term treatment with corticosteroids alone or in combination with α -interferon, or alkylating agents, or azathioprine or high dose immunoglobulins. One case of warm antibody autoimmune hemolytic anemia and one case of chronic Werlhof's disease failed to respond. In one patient with cold agglutinin hemolytic anemia, a complete hematologic remission was achieved, lasting for more than 6 months without any other treatment. In two cases of mixed, type 2 cryoglobulinemia, rituximab administration was followed by the disappearance of purpura and arthralgia. Response duration was 3 months in one case and is not yet evaluable in the other case. In one case of myasthenia gravis, which developed 46 months after an allogeneic bone marrow transplantation, rituximab allowed the dose of prednisone to be reduced from > 0.5 to 0.1 mg/kg/day and that of pyridostigmine from 4 to 2 mg/kg/day. This was a very significant therapeutic achievement, because in this case myasthenia gravis had required intolerable amounts of corticosteroids for many years causing numerous, severe metabolic and infectious complications. In this case rituximab not only allowed a very significant reduction of prednisone, but also substantially improved the neurologic syndrome, with a Karnofsky's performance score that increased progressively from 50 to 90, by 12 months. At the same time, the titer of anti-acetylcholine receptor antibodies decreased from 50 to 20 nmol/L. In all cases CD20⁺ lymphocytes disappeared from peripheral blood for a minimum of 3 months. There were two thrombotic episodes, one affecting the left retinal artery and another affecting the popliteal vein. It was not clear whether they were related to rituximab administration. In conclusion, these preliminary data support larger studies of rituximab treatment in polyclonal and monoclonal immune diseases.

Satellite Symposium
ERYTHROPOIETIN- α IN THE TREATMENT
OF HEMATOLOGIC MALIGNANCIES
(T. Chisesi, F. Dammacco)

ERYTHROPOIETIN IN HEMATOLOGIC TUMORS

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The occurrence of anemia in hematologic tumors usually has several pathogenetic mechanisms, such as bone marrow infiltration, bleeding, vitamin deficiency,

incidence and severity of chemotherapy. Even in the absence of significant activity of these factors, a decreased level of hemoglobin may be the result of an increased production of inflammatory cytokines influencing the differentiation of erythroid precursors (*anemia of chronic disorders*). Most of these factors are common to other malignancies and influence the prevalence of anemia and transfusion requirements. The effectiveness of recombinant human erythropoietin (rhEPO) in preventing anemia and reducing the need for blood transfusion has been suggested and tested in several hematologic disorders including non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia, multiple myeloma and myelodysplastic disorders (MDS). In this presentation special attention will be paid to the use of Epo in lymphoproliferative disorders (non-Hodgkin's lymphoma) (leaving apart multiple myeloma) and in myelodysplastic disorders. *Non-Hodgkin's lymphoma*: More than one third of patients with NHL are anemic (Hb less than 11 g/dL) at diagnosis before the administration of chemotherapy and the severity of anemia increases with the progression of the disease. A potential benefit of Epo administration has been shown in some preliminary non-randomized studies involving heterogeneous groups of patients. Further randomized investigations have shown the capacity of Epo to improve the Hb level, and to reduce the number of transfusions in a consistent number of patients (50-60%). *Myelodysplastic disorders*: The rationale for treating MDS with Epo is mainly related to some biological aspects of these disorders: a) low baseline Epo in 1/3 of the patients, b) persistence in the initial phases of residual erythroid precursors normally responsive to Epo; c) activity *in vitro* of pharmacological doses of Epo on clonal erythroid precursors; d) inhibition of erythroid apoptosis and synergistic action with other growth factors (G-CSF and GM-CSF). The heterogeneity of the responses reported in protocols differing for the clinical features of the patients and therapeutic approaches has suggested that a *response rate* is mainly associated with some defined biological aspects (lower serum Epo levels, recent diagnosis, normal cytogenetics, no significant need of transfusions).

Combining Epo and G-CSF seems to influence the *duration* of the erythroid response. A number of clinical criteria are still under evaluation in order to predict which patients would benefit from this therapeutic approach.

A PLACEBO-CONTROLLED STUDY ON THE EFFECT OF EPOETIN ALFA IN PATIENTS WITH MULTIPLE MYELOMA

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The purpose of this international, multi-center, double-blind study was to evaluate the effect of epoetin alfa (EPO) on transfusion requirements and severity of anemia in multiple myeloma patients on chemotherapy. One hundred and forty-five patients with baseline hemoglobin <11g/dL and at least 6 months on

chemotherapy were enrolled in the study; 132 were eligible for efficacy evaluation. Patients were stratified according to pre-study transfusion dependency and randomized 1:1 to EPO or placebo. EPO was administered at a dose of 150 IU/kg s.c. 3 times a week for 12 weeks (double blind phase) followed by an optional 12 weeks open-label phase. The initial dose of 150 IU/kg was doubled if after 4 weeks of treatment the hemoglobin level had increased less than 1 g/dL above baseline. The study showed that the proportion of transfused patients was significantly smaller in the r-HuEPO group than in the placebo group (28% versus 47%, $p=0.02$) during study months 2 or 3. Of transfusion-dependent patients at baseline, more r-HuEPO-treated patients (48%) than placebo-treated patients (27%) became transfusion independent during study months 2 or 3 (efficacy population, $p=0.03$). In addition, r-HuEPO significantly lengthened the time to first transfusion ($p=0.05$) after 4 weeks on study. Significantly greater increases in both hemoglobin and hematocrit levels (changes from baseline to last value) were observed among r-HuEPO-treated patients than among placebo-treated patients ($p<0.001$). Proportions of "correctors" (Hb ≥ 12 g/dL achieved) and "responders" (an increase of Hb ≥ 2 g/dL over baseline achieved) were markedly higher in the r-HuEPO group (46% and 58%) than in the placebo group (3% and 9%, $p<0.001$). Of patients classified as *responsive* to chemotherapy, more r-HuEPO-treated patients (33%) than placebo-treated patients (0%) showed improvement (i.e. decrease) in performance score. EPO was well tolerated by all patients, as reflected by similar types and frequencies of adverse events between both treatment groups.

In conclusion, the study showed that EPO is a safe and effective treatment for reducing transfusion needs and correcting anemia associated with multiple myeloma in patients on chemotherapy.

GROWTH FACTORS IN HEMATOPOIETIC PROGENITOR CELL MOBILIZATION

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The most significant improvement in hematopoietic progenitor cell (HPC) mobilization has been derived from the observations that hematopoietic growth factors (HGF) can significantly enhance the efficacy of chemotherapy (CHT) alone. HGF are regulatory molecules for proliferation and differentiation of primitive hematopoietic stem cells and lineage-differentiated progenitors cells. The mobilizing effect of G-CSF and GM-CSF alone or after CHT has been documented in several studies and their use has significantly reduced morbidity and costs of autologous stem cell transplantation. The mechanisms of HPC mobilization are still unclear, but experimental studies suggest that migration and homing of stem cells are critically dependent on the interactions

between adhesion molecules in the bone marrow microenvironment (VCAM-1, ICAM-1, fibronectin) and their corresponding ligands ($\beta 1$ and $\beta 2$ integrins, selectins and c-kit). There is some experimental evidence that the association of G-CSF with anti-VLA-4 antibodies significantly increases HPC mobilization in mouse models and that these mobilized HPC show an important down-regulation of SDF-1 receptor.

The most widely used HGF for HPC mobilization is recombinant G-CSF both after CHT and in steady state hematopoiesis, also in healthy donors for allogeneic transplantation. There is general agreement that a threshold number of HPC is required to achieve rapid and sustained engraftment after peripheral blood progenitor cell (PBPC) transplantation. In our center we defined a safe threshold of 2.5×10^6 /kg CD34⁺ cells, while the optimal target ranges between 5 to 7.8×10^6 /kg. A multivariate analysis of collections performed in our center in 182 patients primed with HGF (G-CSF, GM-CSF or G-CSF+erythropoietin) showed failure to obtain a sufficient number of HPC for auto-transplantation in 14.9% of patients; among 156 patients successfully mobilized 72.4% showed optimal CD34⁺ cell collections ($>5 \times 10^6$ /kg), 17.2% safe ($>2.5 \times 10^6$ /kg), 7.4% ranging from 1 to 2.4×10^6 /kg and only 3.1% $< 1 \times 10^6$ /kg. The multivariate analysis of factors affecting the CD34⁺ harvest showed that the only significant factors affecting a *safe* CD34⁺ harvest ($\geq 2.5 \times 10^6$ /kg) were: previous radiotherapy (any site), previous CHT > 6 months and previous failure of priming. In contrast with previous observations we did not observe a significant adverse influence in patients pretreated with fludarabine even though the diagnosis of low grade non-Hodgkin's lymphoma adversely affected only an optimal harvest. In our experience, in accordance with other observations it is possible to identify a small, but not negligible group (15%), of patients who fail even repeated HPC mobilization attempts. These *hard to mobilize patients* require *a priori* identification based both on clinical factors affecting the harvest, and on biological findings such as circulating CD34⁺ cell count at baseline or after a single administration of G-CSF. In this subset of patients alternative strategies for mobilization are needed such as increasing doses of G-CSF, association with other HGF (stem cell factor, erythropoietin) or previous protection of HPC with cytoprotectors (amifostine) before CHT. Our data show a synergistic effect of G-CSF plus erythropoietin after CHT especially in heavily pretreated patients mobilized with DHAP or CTX 7 g/sm² and preliminary data suggest that pretreatment with amifostine can preserve the hematopoietic progenitor content in elderly patients with ANLL, allowing excellent CD34⁺ harvest in 100% of cases.

The burning questions:
CONVENTIONAL CHEMOTHERAPY OR HDS?

**CHEMOTHERAPY OR HIGH-DOSE THERAPY FOR
AGGRESSIVE NON-HODGKIN'S LYMPHOMA?**

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The use of high-dose therapy (HDT) began in the 1980s, when 2nd and 3rd generation regimens were seen to be effective in improving the outcome of advanced stage, aggressive non-Hodgkin's lymphoma (NHL), giving a complete remission (CR) rate ranging from 75% to 85% and a good long-term probability of disease-free survival (DFS). Therefore, the first patients treated were those resistant to conventional chemotherapy or who relapsed after it. Both this first approach and subsequent studies showed that HDT improved CR rate and DFS for these patients, but a first sub-analysis showed that true resistant patients were also resistant to HDT. The problem of chemosensitive relapsed NHL and a possible cure was well established by Dr Philip *et al.* in 1995. HDT was seen to be statistically more effective than conventional chemotherapy in terms of survival and event-free survival. Consequently, patients in partial remission were an interesting category for HDT, but two randomized studies by Dr. Verdonck in 1995 and Dr. Martelli in 1996 did not show any advantage in its use. At the start of the 1990s, several randomized studies (*Gordon 1992, Fisher 1993, NHLCSG 1994*) showed that there was no difference in terms of response rate and outcome between CHOP, 2nd and 3rd generation regimens. From 48% to 56% of patients were expected to be disease-free at 3 years. In 1994, the French Group's study also showed no advantage for patients treated with HDT while in first CR. The use of HDT for aggressive NHL as front line therapy remained to be explored. In 1998, the NHLCSG published a randomized study comparing conventional treatment and HDT in untreated patients. Survival of the two groups of patients was similar with a long-term probability of survival of 65% in both arms. A series of considerations has been made about patients defined as high-risk by the international prognostic index, for whom HDT might improve outcome. However, all results from these studies are retrospective and not randomized so further confirmation is needed. Apart from a particular sub-group of patients (for instance LBL patients), HDT still has not proven itself to be statistically superior to conventional therapy in patients in partial remission, CR or treated at diagnosis. Only chemosensitive patients remain an obvious target for this approach. Perhaps only high-dose *sequential* therapy with or without biological modifiers might change the present situation. A randomized study by the NHL co-operative Study Group exploring the real usefulness of this more aggressive approach is now underway.

6th session
NEW DRUGS IN HEMATOLOGY
(M.J. Keating, M. Baccarani)

**COMBINATION CHEMOTHERAPY STRATEGIES IN
LEUKEMIA AND LYMPHOMA**

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One of the guiding principles of the management of hematologic malignancies is that single agent therapy seldom has a high complete remission (CR) rate or ability to change the natural history of the disease. One exception to this is the use of pentostatin and 2-chlorodeoxyadenosine in hairy cell leukemia. However, in chronic lymphocytic leukemia (CLL) and low grade lymphoma (LGL) single agents have been explored extensively. Chlorambucil has been a major drug in these conditions but has not been associated with a high CR rate in CLL or prolonged remissions in LGL. The advent of the purine analogs, in particular fludarabine (Fludara), have had a major impact on the management of CLL and LGL. This single agent is active in both conditions and is quite possibly the most active single agent in both CLL and LGL. One important element of Fludara is that it inhibits repair of DNA damage caused by agents such as mitoxantrone and cyclophosphamide which cause direct DNA damage. Based on these observations, combination approaches to CLL have included the addition of prednisone to Fludara (FP) or mitoxantrone (FM) or cyclophosphamide (FC). A program has been developed in LGL in which Fludara is combined with novantrone and dexamethasone (FND). In CLL, FP or FM did not improve the CR rate or remission duration and had no impact on survival. FM had no impact on the overall outcome except in previously treated patients in whom survival appeared to be prolonged. However, has improved the CR rate in previously untreated CLL patients and in particular, an alkylator-refractory patients has improved the CR rate and significantly prolonged survival. These results have led to its combination with rituximab for remission induction therapy as frontline treatment in CLL. The FND regimen has been associated with a very high CR rate in follicular lymphomas which have evidence of bcl-2 rearrangement. The FND regimen is a potent salvage programme and has results similar to intensive combination chemotherapy approaches. A number of patients are able to become PCR negative for bcl-2 rearrangement. Thus the era of single agent approaches to management of CLL and LGL appears to be disappearing. Further combination with monoclonal antibodies either simultaneously or sequentially have promised to improve the CR rate even further.

AMIFOSTINE

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The *ideal* chemotherapy and radiotherapy cytoprotectant agent should be safe to administer and should produce a wide spectrum of protection from toxicities without affecting antitumor efficacy.

Amifostine (WR-2721, Ethyol) is a phosphorylated aminothiols and its role in protecting normal tissues from radiotherapy and chemotherapy toxicity has been shown in *in vitro* and *in vivo* studies. In the hematologic setting our *ex vivo* experience shows a selective protection in a model in which amifostine pretreatment protected normal and late progenitor cells from the cytotoxic effects of nitrogen mustard (NM). Indeed the mean LD₉₅ concentrations of NM on CFU-GM (stimulated by GM-CSF and erythropoietin) from 10 patients with acute leukemia or non-Hodgkin's lymphoma was increased from 0.55±0.11 µg/mL with NM alone, to 0.81 ± 0.12 µg/mL in the amifostine-pretreated peripheral blood progenitor cell (PBPC) suspensions. Amifostine pretreatment also protected peripheral blood early progenitor cells from the cytotoxic effects of NM. The mean LD₉₅ concentration of NM on LTC-IC was increased from 0.77±0.18 mg/µL in the control arm to 1.06±0.327 mg/µL in the amifostine pretreated PBPC suspensions. When we tested fresh human leukemia progenitor cells, amifostine pretreatment sensitized the leukemic cells to the cytotoxic effect of NM. The *in vivo* efficacy of amifostine was studied by comparing two groups of 35 and 33 consecutive matched patients, autotransplanted with a conditioning regimen containing high dose melphalan, who received (group A) or not (group B) amifostine (740 mg/m²). Severe mucositis (grade 3-4) was observed in 21% and 53% of cases in group A and group B, respectively ($p=0.006$), its median duration was 0 days (range 0-9) in group A versus 7 days (range 0-11) in group B ($p=0.004$). The duration of analgesic therapy was significantly shorter in group A (0 days; range 0-12), when compared with that in group B (6 days, range 0-20) ($p=0.0001$). We also observed a lower incidence of severe diarrhea (3% vs 25%; $p=0.01$), and emesis (9% vs 34%; $p=0.01$) in group A than in group B. Hematologic recovery, assessed by days to achieve 500x10⁶PMNL and 20,000x10⁶ platelets/L, was not different between the two groups. Moreover a possible role for amifostine in reducing the hematologic and extrahematologic toxicities related to idarubicin has been explored; in our preliminary experience we studied the effect of high-dose idarubicin (40 mg/m²) associated with amifostine in elderly patients affected by high-risk acute myeloid leukemia, in order to reduce the extrahematologic toxicity and the damage of normal bone marrow progenitors, in view of PBPC collection for autotransplantation. Preliminary data showed that this chemotherapy had a very low toxicity in ten patients with a median age of 68 years (range: 57-79). Six out of ten patients (60%) obtained complete remission and we could collect

PBSC for autotransplantation (median CD34+: 7.3x10⁶/kg, range: 4-14.4x10⁶/kg) from 100%; these findings suggest that pretreatment with amifostine before high dose idarubicin reduces extrahematologic toxicity and allows good recovery of PBPC; moreover this latter observation suggests that this phenomenon may be due to selective protection of normal marrow hematopoietic progenitors from idarubicin toxicity, without compromising the response rate.

TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA WITH ORAL FLUDARABINE PHOSPHATE

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Fludarabine phosphate is a nucleotide analog of adenine arabinoside, and its intravenous (i.v.) formulation, Fludara®, has achieved overall response rates of 25-48% in relapsed/refractory B-cell chronic lymphocytic leukemia (B-CLL) in studies carried out in the USA and Europe. An oral formulation of fludarabine phosphate has now been developed and a pharmacokinetic study of single doses in patients with non-Hodgkin's lymphoma and B-CLL showed 24-hour area under the curve (AUC) figures of 2 Fludara® similar to those after i.v. administration, after dose adjustment, with dose-independent bioavailability of 50-60%. From these data it was concluded that a single repeated daily dose of 40 mg/m² would provide a similar systemic exposure to that of a 25 mg/m²/day i.v. dose of fludarabine phosphate. In a multicenter, uncontrolled, open label study oral fludarabine phosphate was administered at a dose of 40 mg/m²/day for 5 days, every 4 weeks, for 6 to 8 cycles, to 78 patients (56 males/22 females) with symptomatic B-CLL (NCI-WG criteria), who had failed to respond or showed signs of disease progression during or after treatment with standard alkylating agent containing regimens (without anthracycline or mitoxantrone). Binet staging at baseline showed 23 A, 24 B and 31 C stage patients. Patients had had a mean of 2 prior treatment regimens. Response to treatment (RR) was evaluated according to IWCLL and NCI criteria. A mean of 5 cycles was administered with 60.3% of patients receiving six or more cycles. Overall RR according to IWCLL was 46.2% (95% CI: 34.8-57.8), including 20.5% complete remission (CR) and 25.6% partial response (PR); according to NCI criteria overall RR was 51.3% (95% CI: 39.7-62.8), with 17.9% CR and 33.3% PR.. Both Binet and Rai disease stages at baseline had a major impact on response, e.g. 29% RR for Binet C versus 58% RR for Binet B. WHO grade 3 or 4 granulocytopenia occurred in 53.4%, thrombocytopenia in 25.6% and anemia in 24.4% of patients. Twenty-five patients required dose reductions, with 20 of these due to myelosuppression. Six infections of grade 3 severity (7.7%) were documented out of a total of 35 reports of infections (44.9% of all patients). Four patients developed autoimmune hemolytic anemia during treatment. Peripheral neurotoxicity occurred in 6.4%. Nausea, vomiting and diarrhea mostly WHO grade 1 and 2 were seen in

38.5%. Four deaths were documented; 2 due to disease progression, 2 due to infectious complications. In general, the efficacy of oral fludarabine phosphate did not differ from that of the i.v. formulation, while the safety profile can be considered acceptable, considering the experience with i.v. Fludara® and the severity of the underlying disease.

CAMPATH-1H ADMINISTRATION FOR THE TREATMENT OF B-CHRONIC LYMPHOCYTIC LEUKEMIA AND LOW GRADE NON-HODGKIN'S LYMPHOMAS

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In B-cell chronic lymphocytic leukemia (B-CLL) and low-grade non-Hodgkin's lymphoma (LGNHL) eradication of the neoplastic clone, with conventional treatment, is extremely difficult. Investigators are, therefore, still searching for new therapeutic agents, including monoclonal antibodies. Campath-1H is a humanized monoclonal antibody targeted against the CDw52 membrane antigen of lymphocytes, which causes complement and antibody-dependent cell-mediated cytotoxicity. Thus, it is much more specific against neoplastic lymphocytes than conventional chemotherapeutic agents. Campath-1H has been used in B-CLL, T-prolymphocytic leukemia (T-PLL) and LGNHL. Campath-1H is administered i.v. thrice weekly for up to 12 weeks. The initial dose is 10 mg, escalated to 30 mg per infusion. The responses [complete (CR) and partial (PR)] that have been reported in untreated B-CLL patients are in the order of 90%. In a multicenter phase II trial of 29 patients with previously treated B-CLL, the response rate was 42%, with 4% CRs. Responses were more prominent in the blood compared to in the lymph nodes. The median response duration was 12 months. These results were verified in a subsequent multicenter study of 92 patients, who had failed to respond to both alkylating agents and fludarabine (overall response 33%, CR 2%, median response duration 9+ months). Because of the antibody's higher activity on circulating lymphocytes it has been used quite successfully for *in vivo* purging of residual disease in CLL, followed by autologous stem cell transplantation. In a multicenter clinical trial of 50 patients with heavily pretreated advanced stage LGNHL, CR was achieved in only 14% of cases with a B-phenotype. A higher response rate (50%) was noted in mycosis fungoides. The CR rate in T-PLL is approximately 60% compared to the 12% CR achieved with deoxycoformycin. Good results have been reported in a small number of patients with refractory autoimmune thrombocytopenia manifesting lymphoproliferative disorders. The main complications of treatment with Campath-1H are caused by tumor necrosis factor- α and interleukin-6 release, usually during the first intravenous infusion, and include fever, rigor, nausea, vomiting and hypotension responsive to steroids. These side effects are usually less severe with subsequent infusions and can be prevented by

paracetamol and antihistamines. Immunosuppression due to normal B- and T-lymphocyte depletion is frequent, resulting in opportunistic infections, mainly in heavily pretreated patients, particularly if purine analogs are included. More clinical trials in larger number of patients are necessary to determine the exact role of and indications for Campath-1H in lymphoproliferative disorders.

A REVIEW OF ZEVALIN RADIOIMMUNOTHERAPY FOR B-CELL NON-HODGKIN'S LYMPHOMA

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Zevalin (IDEC-Y2B8, ibritumomab tiuxetan) radioimmunotherapy (RIT) is under clinical development as an outpatient treatment for B-cell non-Hodgkin's lymphoma (NHL). Zevalin is an anti-CD20 murine IgG1 kappa monoclonal antibody conjugated to a linker (MX-DTPA) that can securely chelate indium-111 (^{111}In) for dosimetry or yttrium-90 (^{90}Y) for therapy. Tumor targeting by zevalin is optimized by pretreatment with rituximab (IDEC-C2B8, Rituxan) to clear the blood of normal and malignant B-cells. Clinical trials of zevalin RIT for relapsed or refractory B-cell NHL are limited to patients with <25% bone marrow involvement, no prior bone marrow or stem cell transplant, and no prior RIT. Toxicity has been primarily hematologic, transient and reversible. A phase I/II trial established the maximum tolerated dose to be 0.4 mCi/kg for patients without thrombocytopenia (platelets >150,000) and 0.3 mCi/kg for those with mild thrombocytopenia (platelets 100,000-149,000) (*Witzig TE: J Clin Oncol 17:3793, 1999*). The overall response rate (ORR) in the phase I/II trial was 67% for all patients (n=51) and 82% for those with low grade NHL (n=34). For responding patients treated at the 0.4 mCi/kg dose level, the median time to progression and duration of response were 15.4 and 14.4 months. Phase II and III trials for patients with low grade, follicular, or transformed NHL have produced encouraging interim results. A phase II trial of reduced-dose (0.3 mCi/kg) zevalin RIT for patients with mild thrombocytopenia demonstrated an interim ORR of 68% (n=22) (*Witzig TE: Blood 94:400a, 1999*). A phase III non-randomized controlled trial of 0.4 mCi/kg zevalin RIT for rituximab-refractory patients produced an interim ORR of 46% (n=24) (*Gordon LI: Blood 94:396a, 1999*). A phase III randomized controlled trial comparing 0.4 mCi/kg zevalin RIT to a standard course of rituximab immunotherapy (375 mg/m² weekly x 4) demonstrated an interim ORR of 80% for zevalin compared to 44% for rituximab (n=90) (*p<0.001*) (*Witzig TE: Blood 94:2805a, 1999*). Grade 4 neutropenia occurred in 25% and grade 4 thrombocytopenia in 6% of the patients receiving zevalin. None of the 179 zevalin patients evaluated with dosimetry has exceeded protocol-defined maximum allowable doses to uninvolved organs (<2000 cGy) or red marrow (<300 cGy). Only 1% developed HAMA or HACA. These results suggest that zevalin RIT offers safe and effective treatment for patients with relapsed or refractory low-grade, follicular or transformed B-cell NHL.

DAUNOXOME. NEW PERSPECTIVES IN HEMATOLOGY

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Daunorubicin (DNR) was the first anthracycline used for the treatment of acute leukemia (AL). However the use of DNR is limited by toxicity and by a mechanism of resistance that develops frequently and quickly also in AL. The mechanism is based on overexpression of a 170 Kd transmembrane glycoprotein (P170 or Pgp) that pumps the drug out of the cells. Pgp overexpression is currently recognized as a major factor of treatment failure, especially in ANLL, in which it is frequently associated with advanced age, a prior history of a myelodysplastic syndrome (MDS), and complex cytogenetic abnormalities. Daunoxome (DNX) is a combination of DNR with a liposomal targeting system, the citrate salt of DNR being entrapped within the inner aqueous core of a small liposome. The liposome prevents DNR deposition and concentration in non-tumoral tissues, so that the toxic profile of the drug is substantially less than that of free DNR. Less toxicity was one reason that stimulated the study of DNX in AL. A second reason was based on the expectation that the liposome entrapped drug would be protected from Pgp and would be concentrated more than free DNR in multidrug resistant (MDR) cells. Based on these premises, DNX was first studied *in vitro*, using several pairs of Pgp-low and Pgp-high cell lines in which it was found that DNX was as toxic as free DNR in Pgp-low cell lines, while it was always 4 or 5 more toxic than free DNR in Pgp-high MDR cell lines. The same results were obtained testing the leukemic blast cells from a number of cases of AL. *In vivo* we investigated first the effect of DNX alone, given at a dose of 60 mg/m² every other day to eleven patients with refractory or very advanced AL. Two patients achieved a short lasting complete remission (CR), and non-hematologic toxicity was remarkably low. In subsequent studies DNX was associated with arabinosyl cytosine (AraC). In one study DNX was given 3 or 4 times at 24 hours intervals, at a dose of 80 mg/m², in combination with a standard dose of AraC (100 mg/m²/day as a continuous i.v. infusion for 7 days). The inclusion criteria were ANLL, all FAB subtypes but M3, age more than 60 years, and no prior treatment. So far, 70 patients have been enrolled in this multicenter GIMEMA study, with a CR rate of 55%. A second study was addressed at patients with very poor risk advanced ANLL, using three doses of DNX with 5 high doses of AraC (2000 mg/m² each). The daily dose of DNX was 80 to 120 mg/m². A CR has been obtained in 10/21 cases or 48%. Three patients (14%) died during induction. Grade 2 or 3 (WHO) gastrointestinal toxicity was seen in 3 patients (14%). Infections occurred in 12 patients (57%). Time to hematologic recovery was 24 days (range 16 and 34) for neutrophils more than 0.5×10⁹/L and 30 days (range 16 and 42 days) for platelets more than 20×10⁹/L. The same protocol of high dose AraC and DNX was used in 10 cases of poor risk adult ALL in 1st early relapse or in 2nd relapse. Nine patients, includ-

ing 3 cases of Ph⁺ ALL, achieved CR after one course. In conclusion, DNX is an effective agent for the treatment of advanced AL, in combination with AraC, and can be administered at an effective dose even to heavily pretreated patients. DNX can become the first line drug for AL in the elderly.

OXALIPLATIN IN NON-HODGKIN'S LYMPHOMA: A NEW THERAPEUTIC ALTERNATIVE?

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Platinum-based agents have a limited role in the management of hematologic malignancies, being mainly involved in salvage regimens (DHAP, ESAP) for patients with non-Hodgkin's lymphoma (NHL). Recently, a third original platinum analog – oxaliplatin – has been approved in Europe for the treatment of colorectal cancer. Preliminary results, obtained during its initial development suggested a potential role for this agent in the treatment of NHL.

Oxaliplatin is the first platinum derivate of the DACH family to be registered. Its clinical development has clearly established its differential spectrum of toxicity when compared to the other available platinum compounds, ie cisplatin and carboplatin. As a single agent, it has no renal or otological toxicity and only minor hematologic toxic effects; neurotoxicity has also shown a distinct clinical profile. In phase II/III trials, antitumoral activity has been confirmed against colorectal and ovarian carcinoma in both front line and salvage therapy.

In a pilot study recently reported, single agent oxaliplatin (100-130 mg/m², q3 wks) was proposed to 22 patients as salvage therapy for refractory/relapsing NHL of heterogeneous histology [follicular lymphoma: 8, diffuse large cell: 3, mantle cell lymphoma: 5, MALT: 2, others: 4]. All patients were pretreated with at least one regimen including an alkylating agent while 18 had received anthracyclines. At inclusion, 63% were considered refractory, their disease having failed to respond to the last therapy. A total of 144 cycles were given, with a median number of 6 (1-30) per patient. Toxicity was modest, limited mainly to reversible grade 1-2 neurotoxicity. Eight objective responses (1 complete remission, 7 partial responses) were observed (40%) in patients with mantle cell lymphoma (3/5), follicular lymphoma (4/8), and MALT (2/2). Noteworthy, one patient with cisplatin refractory disease – he had failed to respond to a DHAP regimen – responded to oxaliplatin, thus supporting the *in vitro* demonstration of partial/non-cross resistance of such platinum derivatives. Of note, the median duration of response was 27 months (range 7-44). Tolerance was excellent, with treatment-related toxicity limited to grade 1-2 nausea/vomiting and reversible grade 1-2 peripheral neuropathy in most of the patients. Finally, these very preliminary results suggest that oxaliplatin should be considered for further development in NHL. Activity needs to be confirmed in formal phase II studies focusing on distinct histologic entities. Only then should new oxaliplatin-based regimens be explored.

IGEV CHEMOTHERAPY AND CONSOLIDATION WITH PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR REFRACTORY-RELAPSED HODGKIN'S DISEASE

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Purpose. Patients with relapsed/refractory Hodgkin's disease (HD) carry a poor prognosis under current standard chemotherapeutic salvage regimens. In this setting, our objective was to determine the efficacy of a chemotherapy with IGEV (ifosfamide, gemcitabine, vinorelbine and prednisone), supported by granulocyte colony-stimulating factor (G-CSF) for cytoreduction and stem-cell mobilization in transplant eligible patients with primary refractory or relapsed HD.

Patients and Methods. Eighteen patients (11 males/7 females) have been accrued between 10/98 and 4/2000. Ten had received two or more prior chemotherapy regimens, and eight radiotherapy as well (three limited and five extended-field). Their median age was 30 years. At accrual, twelve patients had stage III-IV disease or multiple extranodal sites of involvement. Six patients had primary refractory, and twelve relapsed disease. The program consisted in the administration of four cycles of IGEV chemotherapy (ifosfamide 2,000 mg/m²/d iv on days 1 to 4; gemcitabine 800 mg/m²/d iv on days 1 and 4, vinorelbine 20 mg/m²/d iv on day 1, prednisolone 100 mg/d iv on days 1 to 4, and G-CSF 5 µg/kg sc on days 7 to 13) at 3-week interval as induction, provided the evidence of at least partial remission after second cycle. Responding patients (partial (PR) and complete remission (CR), minimal response) then proceeded to autologous or allogeneic PBSC.

Results. Twelve patients are assessable for response. After 4 cycles of IGEV there were six CR and four PR with an overall response rate of 83%, and 2 failures. All patients had peripheral blood stem cells (PBSC) collected, except one who did not undergo PBSC mobilization as he was a candidate for an HLA-matched-sibling non-myeloablative allotransplant. PBSC were collected after cycle 1 in five patients and after cycle 3 in the others patients. All patients started PBSC collection on day 11 or 12 after IGEV therapy. The median number of CD34⁺ cells collected was 7.5 x10⁶/kg (range 3.0x10⁶/kg) after a median of two (range 1 to 3) apheresis procedures. There was no treatment-related hospitalization or toxic deaths. Grade III-IV neutropenia occurred in 52% of 47 evaluable cycles; thrombocytopenia below 50,000 occurred in 25% of cycles with only one patient requiring platelet transfusion. Grade III-IV anemia occurred in 21% of courses with one patient transfused with two red blood-cell units. Only four courses were delayed because of hematologic toxicity. There were minimal non-hematologic side effects. Eleven patients underwent high-dose therapy and transplant. Of four patients in PR after IGEV chemotherapy, three reached CR and in one response was not changed by transplant procedure.

Of nine patients in CR after transplant only one relapsed and obtained a second CR with radiotherapy. At present, with a median follow-up of 8 months (range 2-25) nine patients (75%) are alive and disease-free, two are alive with disease and one died in CR for causes probably related to transplant toxicity.

Conclusions. IGEV chemotherapy is a very effective cytoreductive and mobilizing regimen with acceptable toxicity in patients with resistant/relapsed HD. Further accrual is ongoing.

7th session
MULTIPLE MYELOMA
(M. Boccadoro, R. Bataille)

MYELOMA-INDUCED BONE CHANGES. ROLE OF BISPHOSPHONATES

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Bone destruction is a hallmark of multiple myeloma (MM). Osteolytic lesions are the consequence of increased osteoclastic resorption in the close vicinity of myeloma cells. Quantitative histology has shown that myeloma-induced bone changes are characterized by the stimulation of both recruitment of new osteoclasts and activity of single osteoclasts. Of interest, this process is inhibited by bisphosphonates. Randomized studies have shown that these drugs, mainly given intravenously, could significantly limit the occurrence of skeletal-related events and, in some subsets of patients, improve survival. Further investigation of the mechanisms of action of bisphosphonates has shown (i) that they could reduce the production of myeloma-cell survival and growth factors (e.g., interleukin-6) by bone cells and (ii) that some of them induce apoptosis of myeloma cells. In conclusion, these data indicate that beyond inhibition of bone resorption, bisphosphonates could have true anti-tumoral effects in MM, as in bone metastasis.

CHEMOTHERAPY FOR MULTIPLE MYELOMA

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Chemotherapy for multiple myeloma, namely melphalan and prednisone (MP), was introduced about 30 years ago and sharply improved the natural history of the disease: 40-50% of patients show an objective response, and survival is about 3 years. Combination chemotherapy was then introduced in the early 1970s. Combined use of non-cross-resistant drugs was presumed to be more effective than melphalan alone. A meta-analysis was performed on 18 published trials that contained 3,814 patients who were

randomized to receive either MP or various combination therapy as induction treatment. The main conclusion of this very large study was that the two are equivalent treatments.

In recent years, high-dose chemotherapy (HDT) followed by stem cell reinfusion has been widely used. The superiority of HDT versus conventional chemotherapy was demonstrated by a French randomized trial and other case-matched control analyses. Median remission duration and overall survival are prolonged to 3 and 5 years, respectively. Moreover, peripheral blood progenitor cell (PBPC) support strongly reduced mortality and morbidity of this procedure. Most of the patients treated so far with HDT are under 60 years old. Age at presentation is around 70 years. HDT remains the therapy of choice but applicable to a minority of younger patients. Recently, we presented an intensified approach with a reduced L-PAM dose of 100 mg/m² (MEL100 protocol): patients received 2 or 3 courses of MEL100 followed by PBPC support (*Blood*, 1999). Clinical outcome was compared with that of pair mates selected from patients treated at diagnosis with oral MP and matched for age and β 2 microglobulin. Complete remission was obtained in 47% after the intensified approach and in 5% after MP. The median event-free survival was 34 versus 17.7 months; median overall survival was 58 months versus 46 months. Thus, the intensified approach was clearly superior to standard MP in elderly myeloma patients. This approach has been recently extended to patients up to 75 years with similarly favorable results.

These observations suggest that high-dose or intensified chemotherapies are at present the first therapeutic options for myeloma patients.

ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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In multiple myeloma (MM), high remission rates and survival are obtained with autologous transplantation. However, there is no evidence of cure with this modality, and disease recurrence occurs within 3 to 5 years in most cases. By contrast, relapse rate is low following sibling allograft, but at the expense of a high mortality. In an EBMT analysis, autologous transplantation, despite an increased relapse/progression rate proved superior to the allogeneic strategy in terms of overall survival, due to higher (41% vs 13%) transplant-related mortality (TRM) in the allogeneic group. In order to reduce TRM in sibling transplantation we have used peripheral blood (PBSC) instead of marrow as the source of stem cells. We present here the results in 27 patients. The clinical data are complemented by a study of IgH gene rearrangement for minimal disease assessment.

Transplants were undertaken between 1995 and 1999. There were 22 males and 5 females, aged 31 to 55 years (median 47). Time to transplantation was 3-107 months (median 8). Number of prior chemotherapy regimens varied from 1 to 4 (median 1). Most patients (N=22) were conditioned with busulfan and melphalan. PBSC were collected from HLA-identical sibling donors after G-CSF or sequential GM-CSF and G-CSF. All received unmanipulated grafts containing 4.4 to 24.1 x 10⁶/kg CD34+ cells (median 7.9) and 0.9 to 7 x 10⁸/kg CD3+ cells (median 2.2). GVHD prophylaxis was a combination of methotrexate and cyclosporin A in all. All patients engrafted, with 12 days (range 9 to 17) to achieve an ANC > 0.5 x 10⁹/L, and 11 days (range 11 to 22) to a platelet count >50 x 10⁹/L. Acute GVHD \geq 2 developed in 14 (52%) patients, but it was grade 3-4 only in 4 (15%); chronic GVHD developed in 11 out of the 20 evaluable patients (55%). Following the allograft, 19 (79%) out of the 24 patients evaluable for response were in CR, 4 in PR and 1 proved refractory. TRM was 28%. Disease relapse occurred in a single case. Probability of survival and progression-free survival at 60 months was 66% and 52%, respectively. PCR for IgH-gene rearrangement was available in 11 patients. Two of them died too early while in molecular remission. Another patient was in PR showing only extramedullary disease: he was PCR-negative at 3 subsequent controls, and became PCR-positive a year after transplantation when overt bone marrow progression ensued. In one case the results of PCR assay paralleled the slow reduction of M component following transplantation. In fact, this patient entered CR and became PCR-negative as late as a year after transplantation, at a time when chronic GVHD developed. Five of 10 patients had at least two consecutive PCR-negative follow-up samples and none of them has relapsed so far. Allogeneic transplantation with growth factor-primed PBSC from HLA-identical sibling donors is a valuable option in patients with multiple myeloma, and may offer a high rate of remission with low recurrence rate. These results are accompanied by a profound suppression of the neoplastic clone, as demonstrated by the PCR analysis of Ig-gene rearrangement, presumably mediated by an alloimmune reaction. We suggest transplanting patients early in the course of disease, to avoid undue toxicity and to circumvent the possible emergence of drug-resistance.

MULTIPLE MYELOMA: NEW FRONTIERS IN ALLOGENEIC BONE MARROW TRANSPLANTATION

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Complete remissions in multiple myeloma patients treated at the diagnosis with allogeneic bone marrow transplantation (allo-BMT) or autologous stem cell transplantation (ASCT) are similar (48% v 40%; $p = 0.12$). Transplant-related mortality is, however, higher in allo-BMT than in ASCT (41% v 13%; $p = 0.0001$),



DETERMINATION OF THE DRUG RESISTANCE OF PATIENTS WITH MALIGNANT LYMPHOMA *IN VITRO*

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The phenomenon of resistance to chemotherapeutic agents may be an important reason for ineffective chemotherapy in cancer patients. In this study we evaluated the relationship between predictive markers associated with selective and broad forms of drug resistance (bcl-2, p-53, P-glycoprotein, CD-95), *in vitro* response, and prospective clinical outcomes. Predictive markers were evaluated with immunocytochemistry and flow cytometry. Malignant cells from patients with non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL) were assayed for *in vitro* drug sensitivity in a short-term culture system using the differential staining cytotoxicity (DiSC) assay and the tetrazolium salt based assay (MTT). Drugs used in the treatment of NHL and CLL (prednisone, vincristine, adriamycin, cyclophosphamide, chlorambucil, cytarabine, fludarabine, procarbazine) were tested. We examined 39 patients: 10 with NHL, 29 with CLL. Preliminary data evaluating expression of markers predictive of drug resistance and *in vitro* drug response illustrated that Pgp overexpression was related to a decrease in activity of prednisone, vincristine, doxorubicin, and chlorambucil. Bcl-2 positive leukemia cells, generally associated with a relatively poor prognosis, were significantly more resistant to prednisone, vincristine, doxorubicin than Bcl-2 negative cells. There was a significantly greater percentage of effective drugs in combination in the group of patients who responded to treatment ($76.0 \pm 7.0\%$) than in those, who failed to achieve remission after chemotherapy ($15.8 \pm 5\%$, $p < 0.05$). In conclusion, additional studies to evaluate the clinical value of combining *in vitro* drug-resistance analysis with predictive determinations should be performed.

CD38 EXPRESSION PREDICTS POOR CLINICO-BIOLOGICAL FEATURES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

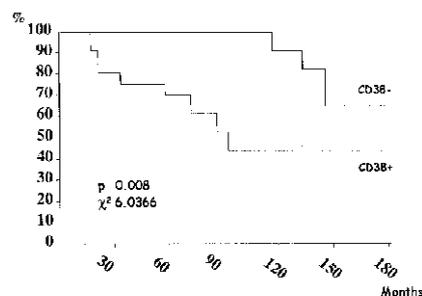
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Human CD38 is a nonlineage-restricted type II transmembrane glycoprotein with a quite widespread cellular expression and functional activity. Attention has recently been focused on CD38 expression in B-cell chronic lymphocytic leukemia (B-CLL). In fact, a recent investigation showed that CD38 was closely associated with unmutated or germline IgV genes (Damble *et al*, *Blood* 1999; 94:1840). Moreover, IgV gene

mutations are strongly correlated with a good clinical outcome and a longer survival (Hamblin *et al*, *Blood* 1999; 94:1848). As a consequence, B-CLL cases can be divided into two categories according to IgV gene mutation status (or CD38 expression); one arising from the expansion of antigen-inexperienced *virgin* lymphocytes, the other from previously triggered post-germinal center *memory* B-cells.

We analyzed the clinico-biological features of 61 immunologically typical (CD5⁺ CD23⁺) B-CLL patients stratified according to CD38 expression. Twenty-two (36%) patients expressed CD38 in more than 30% of CD19-positive cells. In addition, atypical morphology ($p = 0.02$), peripheral blood lymphocytosis ($p = 0.01$) and diffuse histopathologic bone marrow pattern ($p = 0.003$) were found to be closely associated with CD38 expression. However, A and B Binet stages ($p = 0.02$) and interstitial bone marrow involvement ($p = 0.005$) were more represented in the CD38-negative B-CLL group. Finally, median survival of CD38-positive B-CLL patients was 90 months, while median survival had not been reached at 180 months in the CD38-negative patients. Taken together, our data suggest that the expression of CD38 may identify two groups of chronic B-lymphoid tumors differing in their clinico-biological features.



EVALUATION OF MINIMAL RESIDUAL DISEASE IN LOW GRADE B-CELL NON-HODGKIN'S LYMPHOMA: PROGNOSTIC SIGNIFICANCE AND TECHNICAL PROBLEMS. A SINGLE CENTER EXPERIENCE

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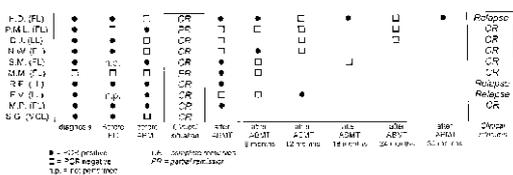
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The real importance of the discovery of minimal residual disease (MRD) after therapy and which is the best molecular marker to evaluate it are still open questions. The aims of this study are to determine the presence of MRD both after conventional therapy and after autologous bone marrow transplantation (ABMT) in low grade B-cell non-Hodgkin's lymphoma (B-NHL), and to clarify the role of the

immunoglobulin heavy gene (IgH) rearrangement as a diagnostic tool in B lymphomas.

From 1996 to 1999 we analyzed 84 patients (48 male, 36 female, median age 62 years) with low grade B-NHL. According to the R.E.A.L. classification, 44 patients had follicular lymphoma (FL), 21 lymphocytic lymphoma (LL), 11 mantle-cell lymphoma (MCL), 5 hairy-cell leukemia (HCL) and 3 marginal zone B-cell lymphoma. The molecular analysis we used to evaluate MRD was based on the determination of IgH rearrangement through amplification by PCR. We chose the combination of FR2 and FR3A semi-nested methods, that, as has been demonstrated, reveals 85% of monoclonality. PCR analysis was performed on tissue, peripheral blood (PB) and bone marrow (BM) at diagnosis, and on only PB and BM after therapy and during follow-up. A patient is considered PCR positive when one of the samples results positive.

Results. Overall IgH+ rate was 83% (73% in FL and 93% in non-FL). We analyzed 71 patients at diagnosis: IgH rearrangement was identified in tissue, PB and BM samples in 87%, 60% and 72% of the cases, respectively. In FL (39 patients) IgH+ rates in tissue, PB and BM samples were 82%, 41% and 62% respectively, whereas in non-FL (32 patients) they were 95%, 83% and 90%, respectively. Twenty-seven patients received conventional chemotherapy; IgH+ rates were 85% at diagnosis and 44% after therapy. Ten patients (6 FL, 3 LL and 1 MCL) underwent treatment with high-doses of cyclophosphamide followed by ABMT. IgH+ rate was 90% at diagnosis and 55% after ABMT.



In 1998 we started to search for Bcl-2 translocation as a molecular marker specific for FL. Twenty patients with FL were studied at diagnosis and 60% of them resulted Bcl-2+, whereas 12 patients were treated and 25% of them resulted Bcl-2+ after treatment.

Conclusions. Our results demonstrate that the IgH rearrangement is an extremely sensitive method in monitoring MRD in low grade B-NHL. In particular, it is more useful at diagnosis for non-FL, whereas it is advisable to use both IgH rearrangement and Bcl-2 translocation for FL. At diagnosis, the most significant results by using PCR analysis are obtained on tissue; however, it was important for us to perform it both on PB and BM because in some cases (11) we obtained PB+ and BM- after therapy. Conventional therapy and ABMT do not induce a significantly high molecular response. When a follow-up evaluation is performed there is a good correlation between molecular results and clinical outcome. It is then possible to associate the presence of monoclonality after ABMT with an increase of incidence of relapse. In conclusion, the detection of MRD by PCR of IgH rearrangement could

have a prognostic value and a clinical significance, and most of all can be useful to assess the role of ABMT according to the results obtained.

IMPAIRED PHAGOCYTOTIC FUNCTION OF POLYMORPHONUCLEAR NEUTROPHILS IN B CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction. The functional activity of polymorphonuclear neutrophils (PMNs) in patients with B-cell chronic lymphocyte leukemia (B-CLL) is still controversial due to the heterogeneity of the disease and individual variability, although normal absolute numbers of PMNs have been described. We have recently shown¹ a significant ($p < 0.001$) decrease in the ability to release reactive oxygen intermediates in the PMA-induced nitroblue tetrazolium test (NBT) by PMNs of B-CLL patients ($37.9 \pm 19.0\%$, range 12-83%) compared with normal controls ($81.5 \pm 12.7\%$, range 59-94%). Both, the number of NBT+ PMNs and the intensity of the formation of formazan crystals were decreased.

Methods. The detection of immature PMNs was measured by the activity of leukocyte alkaline phosphatase (LAP) using a Sigma LAP kit in 10 untreated Rai staged patients (age range 48-77) and 10 age-matched controls. PMN were analyzed morphologically to determine banding. A possible overall decrease in enzymes, related to oxygen-dependent bactericidal function was evaluated by the expression of myeloperoxidase (MPO) in PMNs using a cytochemical assay (Sigma). The ability of PMNs to attach and engulf opsonized target cells was also tested. For this purpose, *Staphylococcus aureus* were opsonized with specific rabbit polyclonal IgG antibodies (Molecular Probes) and added to PMNs isolated on double Ficoll gradients. Cells were incubated for 30 minutes at 37°C, washed and stained with Giemsa. The numbers of PMNs with internalized and/or attached opsonized bacteria were evaluated per 200 PMNs.

Results. The intracellular expression of LAP by blood PMNs in B-CLL patients ($67.7 \pm 13.2\%$, range 52-81%) did not differ from that in healthy controls ($71.7 \pm 15.2\%$, range 50-85%), excluding immaturity of the PMNs as a factor. Morphologic analysis confirmed that there were very few band-type immature PMNs in the blood of B-CLL patients. The expression of MPO by B-CLL PMNs ($94.3 \pm 1.5\%$) was no different from that of normal controls ($92.7 \pm 1.9\%$), suggesting that the oxygen-dependent peroxidase system was not impaired. Although attachment of the opsonized particles was similar to that in controls, there was, nevertheless, a significant decrease in internalization by B-CLL PMNs (Table 1).

Table 1. Internalization of opsonized *S.aureus*.

Source of cells	PMN with or without <i>S. aureus</i> , %			
	Attached only	Attached and internalized	Internalized only	None
B-CLL patients	36.0±11.1	5.6±5.9	14.3±10.9	42.3±25.3
Controls	37.5±4.0	17.4±8.6	40.2±5.9	4.9±0.8
P	> 0.05	> 0.05	< 0.001	< 0.01

*Mann-Whitney test (the data represent mean ± standard deviation).

Conclusions. Our data are consistent with a reduced phagocytic function of PMNs of B-CLL patients which might contribute to the increased susceptibility to infection in this disease.

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FLUDARABINE IN COMBINATION THERAPY IS AN EFFECTIVE TREATMENT FOR RELAPSED OR REFRACTORY LOW-GRADE NON-HODGKIN'S LYMPHOMA: A MULTICENTER EXPERIENCE

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The aim of our study was evaluate the efficacy and safety of fludarabine in combination with cyclophosphamide or with mitoxantrone and cyclophosphamide in patients with recurrent low-grade lymphoma (LGL). Fifty-three patients entered the study. Twenty-two patients received cyclophosphamide (300 mg/m² i.v. days 1-3) followed by fludarabine (25 mg/m²) in 30 min. infusion days 1-3, every 28 days for a maximum of 6 cycles; 31 patients also received mitoxantrone (10 mg/m² given over 15 min. infusion on day 1. All patients received antibiotic prophylaxis and growth factors (G-CSF) if grade III granulocytopenia (WHO) occurred. All patients had failed a median number of 2 (range 1 to 5) previous chemotherapy regimens containing either doxorubicin or mitoxantrone. Mean age was 56 years (35-75). According to the REAL classification, 12 patients had small B lymphocytic, 34 patients follicular, 5 mantle cell and 2 marginal zone NHL. The overall response rate in all patients was 88% (58% with CR). In FLU/CY it was 95% compared with 84% in FLU/CY/MITO. After 3 courses, 58% of overall CR was achieved with FLU/CY treatment and 90% with FLU/CY/MITO ($p=0.02$). There was no statistical difference in response rate between the treatments. Median time to disease progression was 6 months. One patients died with fever of unknown origin 3 months after 6 courses of FLU/CY while in CR. Both therapies were well tolerated. Grade 3 or 4 neutropenia was observed in 39 courses, and had a similar distribution between the two groups. Non-hema-

tologic toxicity was very mild in both arms and represented by grade 1 nausea and vomiting in two patients. No other toxicity was observed. Both combination therapies were seen to be effective in treating recurrent low-grade lymphoma in patients previously treated with regimens containing doxorubicin or mitoxantrone, but fewer patients relapsed with FLU/CY/MITO and the fast activity of this treatment suggests it to be more useful. Overall results were similar, consequently it is difficult to draw sure indication about the opportuness of choosing one or the other treatment.

CIS-PLATINUM, IDARUBICIN, PREDNISONE AS CONSOLIDATION THERAPY AFTER P-VABEC CHEMOTHERAPY FOR ELDERLY PATIENTS WITH DIFFUSE LARGE LYMPHOMAS. AN ITALIAN MULTICENTER RANDOMIZED STUDY

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Background. In a phase II study the P-VABEC regimen resulted to be an active and well tolerated therapy for elderly patients with diffuse large cell lymphomas (Martelli, *J Clin Oncol* 11:2363; 1993). Further studies reported on a larger series of patients treated with P-VABEC demonstrated that in spite of a high rate of complete response (CR) the event-free survival (EFS) rapidly decreased with a high incidence of early relapse. Moreover a significantly worse EFS was shown for patients with a high-risk IPI score compared to those with a low-risk IPI (Martelli, *Ann Oncol* 1999; 10 suppl. 3, 55). A phase II study reported by Caracciolo (*Leuk Lymphoma* 1997; 24:335) demonstrated an improvement of survival after cisplatin, idarubicin, prednisone (CIP) as consolidation chemotherapy in patients responsive to P-VABEC.

Purpose. To evaluate the activity and toxicity of CIP consolidation therapy after P-VABEC versus the standard P-VABEC regimen in a prospective, randomized, phase III study.

Patients and methods. From October 1995 to April 2000 we enrolled 198 previously untreated patients with diffuse large cell lymphomas (according to the REAL classification), median age 70 years (range 60-85), stage II-IV. All eligible patients were randomized at diagnosis to receive P-VABEC (group 1) or P-VABEC-CIP (group 2). The P-VABEC is an 8 weekly regimen delivered on a out-patient basis: doxorubicin 30 mg/m², etoposide 100 mg/m², cyclophosphamide 350 mg/m² given in weeks 1, 3, 5, 7 and vincristine 1.4 mg/m², bleomycin 15 mg/td in weeks 2, 4, 6, 8. A daily prednisone dose of 50 mg is given orally during the entire regimen. The CIP consolidation therapy started 21 days after the last P-VABEC cycle, in patients who obtained a response (complete or partial). Patients with minimal response or progressive disease after P-VABEC were excluded from consolidation therapy. The CIP schedule 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. So far 160 patients are evaluable for response, 90 in P-VABEC (group 1) and 70 in group 2. According to the age-adjusted IPI, 81 patients were considered as low risk (IPI 0-1) and 79 as high risk (IPI 2-3).

Results. With a median follow up of 24 months (range 1-54) the CR rate, overall (OS) and EFS at 2 years were 63%, 58%, 54% in group 1 and 62%, 72%, 68% in group 2 ($p=ns$). No severe toxicity has been reported for the CIP regimen except for a moderate increase of peripheral neurotoxicity. There were 8/160 (5%) toxic deaths related to chemotherapy, all occurring during the P-VABEC chemotherapy.

Conclusions. The CIP regimen, given on an out-patients basis, has been a safe and well tolerated chemotherapy in elderly patients. According to our preliminary results, CIP consolidation therapy does not significantly improve the survival of elderly patients previously treated with P-VABEC. The study is still ongoing and an additional update of results will be presented.

EBV-NEGATIVE LYMPHOPROLIFERATIVE DISORDERS IN LONG-TERM SURVIVORS OF HEART, KIDNEY OR LIVER TRANSPLANT

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Post-transplant lymphoproliferative disorders (PTLD) can be defined as polyclonal or monoclonal lymphoproliferations mediated by the Epstein-Barr virus (EBV) occurring in 1% to 10% of patients within the first or second year after a solid organ transplant (SOT) or allogeneic bone marrow transplant (BMT). However, SOT patients undergoing long-term immunosuppressive treatment to prevent graft rejection, still have a high risk of developing lymphomas as compared to general population. The characteristics and the pathogenesis of these late occurring PTLD have not yet been extensively investigated. We studied 15 patients who developed PTLD 79 months (range 22-156 months) after SOT (12 heart, 2 kidney and 1 liver). The histologic diagnosis of 17 specimens was as follows: polymorphic-PTLD in 3 cases (17%) and monomorphic-PTLD in 14 cases (83%). In particular, within the monomorphic-PTLD we recorded: diffuse large B-cell lymphoma (DLCL) in 9 cases (53%), anaplastic large cell lymphoma (ALCL) in 1 case (6%), multiple myeloma-like (MM-like) in 2 cases (12%) and Burkitt's lymphoma-like (BL-like) in 2 cases (12%). The immunophenotype showed B-lymphoid proliferation in all samples except for the ALCL that resulted negative for both B and T-lymphoid markers. Clonality, presence of EBV genome and genetic lesions were evaluated by Southern blot analysis or polymerase chain reaction (PCR). All monomorphic-PTLD and two of three polymorphic-PTLD showed a monoclonal pattern of the heavy chain immunoglobulin (IgH) rearrangement.

Overall, 44% of samples demonstrated the presence of the EBV genome. Within monomorphic-PTLD the percentage of EBV-positive lymphomas was even lower (31%). C-myc gene rearrangements were demonstrated in two cases (13%). None of the 15 samples showed bcl-1, bcl-2 or bcl-6 rearrangements. Although, there is neither a uniform nor a standard approach for treatment of PTLD, the reduction of immunosuppression is recommended as first line treatment and chemotherapy is the most frequent option offered to patients unresponsive to immunosuppression discontinuation. In our series, the modulation of immunosuppression was ineffective in all patients with monomorphic-PTLD and independent of the presence of the EBV genome. The clinical outcome after chemotherapy was poor due to infectious complications and resistant disease. With a median follow-up of 4 months, the median survival time of these patients was 7 months. In conclusion, late occurring lymphomas can be considered a distinct entity from PTLD occurring within one year after transplant because they show a histologic and clinical presentation similar to lymphomas of immunocompetent subjects, are frequently negative for the EBV genome, are invariably clonal and may rearrange the c-myc oncogene. New therapeutic strategies are required to reduce the mortality rate.

FLUDARABINE PLUS CYCLOPHOSPHAMIDE IS AN EFFECTIVE REGIMEN FOR PATIENTS WITH PRETREATED CHRONIC LYMPHOCYTIC LEUKEMIA.

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The purine analog, fludarabine (FLU), produced promising results in the treatment of both recurrent advanced low grade non-Hodgkin's lymphoma and B-chronic lymphocytic leukemia (B-CLL). The unique mode of action of FLU, which affects DNA and RNA synthesis, including DNA repair, has opened up the possibility of using this new agent in combination to potentiate the effect of other drugs. Laboratory data have shown FLU to be synergic *in vitro* with cyclophosphamide (Cy). Recently FLU combined with Cy has been studied in clinical trials at the MD Anderson Cancer Center with promising results in previously treated patients with B-CLL. We report the results of a multicenter retrospective study started in June 1997 in pretreated patients with recurrent/relapsed active B-CLL patients. All patients received FLU 30 mg/m²iv infusion daily for three consecutive days combined with C 250y mg/m² iv infusion for three days for a minimum of two up to six courses, every four weeks. Patients are evaluable for response and toxicity if they received at least two courses. Thirty-three patients entered the study (21 males); the median age is 60 years (range 36-76). The median time between diagnosis and treatment was 60 months (1-164); the median number of prior treatments was 2 (1-5); 19

patients had refractory disease; 23 are in intermediate risk stage (Rai I+II) and 10 in high risk (III+IV); the median WBC count is $48.5 \times 10^9/L$ (2.3-180.0).

To date 27 patients have concluded the treatment plan and are evaluable for response and toxicity, according to NCIWP and WHO criteria. There are 4 complete responses and 17 partial (3 good PR + 14) with a global response rate of 77.7%. Six patients (22.3%) are refractory (SD=3, PD=3). Seven deaths (4 because of progression) occurred in the follow-up. Toxicity was moderate and mainly myelosuppression (WHO grade 3) in 10 out of 33 patients. CD4 positive cells, evaluated pre and post transplantation, lowered significantly (about 50%). We conclude that FLU-Cy is a highly effective regimen in salvage therapy for heavily pretreated active B-CLL. Hematologic toxicity is acceptable with considerable immune suppression. Further prospective studies need to confirm our data.

HCV-ASSOCIATED NON-HODGKIN'S LYMPHOMA / LEUKEMIAS AND MALIGNANT IMMUNOSECRETORY DISORDERS IN A SINGLE CENTER : A CLINICO-PATHOLOGICAL STUDY OF 193 CASES

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In the years 1993-1999, 193 cases of non-Hodgkin's lymphoma/leukemia (NHL/L) and malignant immunosecretory disorders (multiple myeloma-MM) were followed by the Hematologic Section of San Giacomo Hospital's Dept. of Internal Medicine in Rome. Previously untransplanted, consecutive patients were investigated for anti-HCV serum antibodies, and 37 cases (19.2%) were found to be HCV+. However, a higher percentage of HCV+ cases was found in the NHL group (24/97 cases, 24.7%) and in the B-CLL group (6/30 cases, 20.0%), than in the MM group (7/66 cases, 10.6%). The virus genotype, and the presence of viral RNA in the serum and in peripheral blood mononuclear cells (PB-MC) were investigated in 18 MCV+ patients. Clinical and follow-up data, including the liver disease status was examined over a period of several years. A retrospective pathological study of NHL/L and MM was performed according to the criteria of the WHO classification of lymphoproliferative diseases. The clinico-pathological features of the HCV+ cases were compared with those of the HCV- cases. HCV+ -NHL were mostly extranodal both in the low- and in the high-grade malignant groups; small B cell NHL were mostly marginal zone lymphomas (MZL), of either MALT- or splenic type.

A NEW PURGING STRATEGY: EX VIVO ANTI CD20 TREATMENT AND IN VIVO PURGING OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Mabthera is a chimeric anti-CD20 monoclonal antibody (MoAb) that selectively binds the CD20 antigen found on the surface of malignant and normal B-cells. Mabthera is the first antibody approved and utilized for the treatment of low grade lymphomas. We are conducting a study on the use of Mabthera for *in vivo* purging of residual B cells contaminating a peripheral blood stem cell (PBSC) graft, after the MoAb had been bound *ex vivo* to the surface of B- lymphocytes before infusion, in B-CLL patients. We report the first case of this study. A 48-year old man with B-CLL (Rai stage II) was treated by fludarabine 25 mg/m^2 for 5 days at 28-day intervals for 4 courses. He achieved normal peripheral blood and bone marrow lymphocyte counts, but immunophenotypic analysis showed that 28.7% of peripheral blood lymphocytes and 35.7% of bone marrow lymphocytes were CD19+ CD5+. He has undergone PBSC mobilization using CTX (3.5 g/m^2 die) and VP16 (300 mg/m^2 die) for 2 days, followed by daily rh-G-CSF ($5 \text{ } \mu\text{g/kg}$ s.c.). PBSC was collected on the 12th day by a Fresenius separator. The apheresis products contained $3.6 \times 10^6/\text{kg}$ CD34+, and 29.7% of the total cells were CD5+ CD19+ (residual B-CLL cells). Conditioning chemotherapy was the BEAM regimen followed by reinfusion of the Mabthera manipulated harvest. In brief: PBSC were rapidly thawed at 37°C and normal saline (volume 1:2) was added drop by drop. After centrifugation (2500 rpm x 15 min) the supernatant was discarded and the pellet resuspended with 100 mL of RPMI1640 medium (containing heparin 10 IU/mL). Mabthera was added (100 mg to each bag) and after a 30' incubation, the cells were washed with RPMI1640 medium. Evaluations on the final material showed a viability of 92% with a recovery of 67% of the total CD34+ cells collected prior to cryopreservation. The patient was premedicated with acetaminophen plus diphenhydramine and the cells rapidly infused ($2.4 \times 10^6/\text{kg}$ CD34+ were finally infused). Hematological recovery was on 16th day (PMN > $500/\text{mm}^3$) and on 24th day (Plts > $50,000/\text{mm}^3$); complications were limited to neutropenic fever and mild mucositis. The patient is now in clinic-hematologic remission 15 months after the transplant with normal white blood lymphocyte count. This is the first report of a new use of Mabthera and a new concept of B-cell purging in B-CLL patients. It is our opinion that this method can be extended to all B-cell CD20+.

BLOOD CLOTTING AND INDICES OF TISSUE DESTRUCTION IN PATIENTS WITH MALIGNANCIES

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We have investigated the linkage between tissue destruction and blood coagulation in patients with malignant disease of: lymphatic tissues and thymus, stomach, esophagus, intestines, lung, breast, skin, soft tissues, connective tissues, uterus, and nervous system (143 patients).

The indices of tissue destruction (which reflect the efflux-fragmentation-membrane rupture) which were determined in the serum or in the plasma were:

1. Level of c-AMP.
2. Content of the electron spin resonance detected paramagnetic centers (ESR – dpc).
3. Values of $^1\text{H-NMR}$ (T_1, T_2) relaxation times.
4. Activity of 5'-nucleotidase (EC 3.1.5.5), 5'-NC.
5. Content of fibrinogen degradation products (FDP).

Morphologic studies (including optical and electron microscopy) were carried out in order to estimate:

1. Morphologic integrity of the blood vessels.
2. Fibrin deposition in the extravascular space.

Results. The number (%) of tissue destruction indices which exceeded the range of normality was the following:

- 1.1. Level of c-AMP-40.9;
2. Content of ESR-dpc – 27.9;
3. Values of $^1\text{H-NMR}$ (T_1, T_2)-2;
4. Activity of 5'-NC-40.9;
5. FDP-61.7.

Despite the fact that epithelium and basement membrane were intact, mature fibrin deposition was observed in the subendothelial connective tissue and in the epithelium. The clinical combination of 5'-NC activity and FDP content could be divided into four groups: 1) 5'-NC-normal, FDP-normal 2) 5'-NC-exceeds normal, FDP-normal 3) 5'-NC normal, FDP-exceeds normal 4) 5'-NC-exceeds normal, FDP-exceeds normal.

Conclusions. 1. Activity of 5'-NC, content of c-AMP, FDP, ESR-dpc and the value of T_1, T_2 reflect the extent of tissue destruction in patients with malignancies. Measurement of $^1\text{H-NMR}$ (T_1, T_2) relaxation times is useful to understand the dynamics. 2. Blood clotting and fibrin deposition in tissues occur even without cells destruction. the early stage of this process is the increase of blood vessel permeability and the contact of plasma with the surface of cells located extravascularly.

SEA BLUE HISTIOCYTOSIS SECONDARY TO NIEMANN PICK DISEASE TYPE B: A CASE REPORT

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Sea-blue histiocytosis is a morphologic finding that can be associated with both hematologic conditions in which an increased cellular turnover is present (eg. myelodysplastic syndromes or myeloproliferative disorders) and inborn errors of lipid metabolism, like Gaucher and Niemann Pick disease (NPD). We report here a case of sea-blue histiocytosis associated with a mild phenotype of NPD type B. A 44-year old Caucasian man sought medical attention because of splenomegaly and mild thrombocytopenia, without other signs or symptoms. Personal and family history were unremarkable. On physical examination only a 7x7x6 cm palpable spleen was found; abdominal US showed homogeneous spleen enlargement, without parenchymal lesions and no abnormalities. Laboratory findings were the following: Hb 15.2 g/dL, leukocytes 5,100/mm³ with a normal differential count, platelets 86,000/mm³, normal erythrocyte sedimentation rate, serum cholesterol and triglycerides were normal, total serum bilirubin 1.5 mg/dL, AST 40U/L ALT 74U/L, GGT 114U/L; serology was negative for HBV and HCV, anti-platelet antibodies were not detectable. Bone marrow biopsy and aspirate showed foamy and sea-blue histiocytes. Measurement of acid lisosomal sphingomyelinase activity figured below normal in two different determinations (1.81 and 4.77 nmol/mg respectively, normal values > 9.85). These findings were pertinent to the diagnosis of NPD type B, mild phenotype. The incidence of NPD type B is probably underestimated: this rare inborn error of metabolism becomes evident during adult age, classically with splenomegaly and thrombocytopenia. Contrary to what happens in NPD type A, it is not associated with neurologic involvement; the course and prognosis are benign. Measurement of acid lisosomal sphingomyelinase activity is a simple and non-invasive test that enables a precise diagnosis when clinical and morphologic findings are pertinent and a primitive hematologic disorder has been ruled out. This case confirmed that a partial sphingomyelinase deficiency (NPD type B) may be one cause of sea-blue histiocytosis.

SECOND THERAPY-RELATED CANCER IN PATIENTS TREATED FOR HODGKIN'S DISEASE: THE CANCER RESEARCH CENTER EXPERIENCE

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Advances in the management of local stages of Hodgkin's disease (HD) have gradually increased over 20 years relapse-free survival in 80% of all patients. Nowadays, 95% of patients remain in complete remission for a median follow-up of 6 years. But these patients are at a very high risk of developing a second malignancy, with a frequency from 2% at 6 years to 5% at 10 years after HD.

Objectives. More than 2300 patients (600 children and

1700 adults) have been treated for HD in the CRC from 1965-1999. The median follow-up period was 8 years (3-29). Second primary cancer was diagnosed in 48 (2%) patients, including 18 ANLL, 27 solid tumors (thyroid, 7; breast, 6; gastric, 5; lung, 3; cervix uterus, 2; synovial and osteogenic sarcoma, melanoma, mesothelioma, 1 each) and 3 lymphomas (MALT-lymphoma - 1, Burkitt's lymphoma - 1, NHL - 1).

We also observed 1 case of Hodgkin's disease which developed in an 8-year old girl with ALL in remission 1 year and 10 months after the end of the BFM-90 protocol. She was treated by combined therapy and is now in remission from both malignant disorders.

Results. There was a significant prevalence of unfavorable forms of HD in the patients who developed ANLL. The treatment program for these patients was more aggressive than for those who developed solid tumors, consisting of combined modalities (93% and 60% respectively) and alkylating agents (87% and 50%). The treatment of all second leukemias was ineffective both in children and adults while a long-term effect was achieved in 16 patients with solid tumors with a follow-up of 9 years in the children and 6.5 years in adults.

Conclusions. Our data confirm the results of large clinical and epidemiological studies of the carcinogenicity of both chemotherapy and radiotherapy and particularly alkylating agents.

CLONAL HEMATOPOIESIS DETECTED BY X-INACTIVATION PATTERN IS ASSOCIATED WITH A HIGH RISK OF THROMBOSIS IN YOUNG FEMALE PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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We performed clonal analysis of hematopoiesis using the X-chromosome inactivation pattern (X-CIP) in 35 female patients, median age 33 years (range 20-63), median platelet count at test $678 \times 10^9/L$ (range 220-1300) with essential thrombocythemia (ET) according to the PVSG criteria. We excluded female patients older than 65 years in order to reduce the presence of age-related skewing. We also assessed the presence of endogenous erythroid colonies (EEC) considered a specific and reliable marker of myeloproliferative disorders. Cytogenetic analysis showed a normal 46XX karyotype and there was no evidence of bcr/abl rearrangement. Ten out of 35 patients (28.57%), median age 29 years (range 20-37) had thrombosis. Seven patients developed splanchnic thrombosis involving the portal vein, mesenteric vein, splenoportal vein and Budd-Chiari syndrome, 2 had recurrent fetal losses and 1 axillary vein thrombosis. The remaining 25 patients (71.42%), median age 40 (range 22-63) did not develop thrombosis at diagnosis or during follow-up. Clonality was assessed on neutrophils, platelets and EECs. We investigated different DNA polymorphisms: HUMARA in nucleated cells and IDS and p55 mRNA in platelets and EECs.

Control tissue from the same patients (T-cells) excluded constitutive skewed X-CIP or age related acquired skewing of X-CIP. Clonal hematopoiesis was found in 15/35 (43%), 13 patients had polyclonal hematopoiesis (37%) and 7 patients were considered uninterpretable due to constitutive skewing (20%). In patients with thrombosis the percentage rose to 90% since 9 out of 10 patients exhibited clonal hematopoiesis. Among patients with polyclonal hematopoiesis 1 patient (7.6%) developed thrombosis of the axillary vein preceding the diagnosis of ET. This patient had prothrombin G20210A as an additional factor risk for thrombosis. Four patients with clonal hematopoiesis treated with low dose aspirin and/or platelet lowering agents have not so far had thrombotic events. Two additional patients with clonal hematopoiesis have not been treated yet and have not developed thrombosis. Clonality was confirmed on purified CD34⁺ subpopulations from bone marrow in 7 patients, documenting that clonality does not appear lineage restricted. EECs were present in 19 out of 32 patients (59.3%). Twelve out of 15 patients with clonal hematopoiesis had EECs (80%) while 4 out of 10 with polyclonal hematopoiesis had EECs ($p=0.08$, Fisher's exact test). EECs plucked from *in vitro* culture of patients with clonal hematopoiesis were found to have a clonal pattern. We compared the clinical features of clonal and polyclonal patients. There were no statistical differences in age at diagnosis, median platelet at test and duration of follow-up. Thrombotic episodes were significantly more frequent in the monoclonal group ($p=0.006$, Fisher's exact test) Young female patients with essential thrombocythemia exhibiting a clonal pattern of hematopoiesis by X-CIP analysis are at high risk of thrombosis particularly in unusual sites. Splanchnic thrombosis may be life-threatening and carries a high percentage of post-thrombotic complications. These data confirmed that ET is a heterogenous disease. X-CIP analysis may help in defining the individual risk and tailoring the approach to treatment, including the use of cytostatic agents and will also allow a correct diagnosis in patients with latent myeloproliferative disorders and thrombosis in unusual sites. Clonal hematopoiesis is easily recognizable by X-CIP but its applicability is limited to the female sex and is hampered by the presence of age-related or constitutive skewing.

BFM-LNH 81 PROTOCOL AS FRONT-LINE TREATMENT FOR CLASSICAL BURKITT'S AND BURKITT-LIKE NON-HODGKIN'S LYMPHOMAS

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Burkitt's lymphomas (BL) account for less than 1% of all diagnosed non-Hodgkin's lymphomas (NHL) in adults. The recently published new WHO proposals for classification of lymphoid neoplasms distinguishes morphologic variants (Burkitt-like with or without plasmacytoid differentiation) and subtypes (endemic, sporadic, and immunodeficiency-associated) of BL. In contrast to classical BL, the morphologic diag-

nostic criteria of Burkitt-like variant are less well defined by virtue of more pleomorphism or large cells than classical BL, which sometimes makes the differential diagnosis with diffuse large-B-cell lymphoma (DLBCL) difficult. The optimal treatment of Burkitt-like NHL still remains an unresolved question. A matter of debate is now whether this variant of BL should be treated with regimens for Burkitt's lymphoma or with those usually used to treat DLBCL. We present here the results and follow-up of 26 HIV negative patients (18 classical BL, 8 Burkitt-like), median age 41 years (range 16-79), treated with the BFM-LNH 81 protocol in the last 12 years. The regimen consisted of alternating administration of Block A (CTX 200 mg/m² IV on days 1-5, MTX 500 mg/m² IV given continuously IV over 24h on day 1, Ara-C 300 mg/m² IV, and VM-26 165 mg/m² IV both on day 5), and Block B which differs from Block A for Adriamycin 50 mg/m² IV on day 5 instead of Ara-C and VM-26) for six cycles every two weeks for patients with stage III-IV and three weeks for those with stage I-II. In order to prevent acute tumor lysis syndrome all pts received CTX 200 mg/m² for five days only before the first Block A. All but three patients received CNS prophylaxis with MTX 12 mg td IT on day 1 of each course. Involved field radiotherapy (IF-RT) was delivered to residual masses or previously bulky sites. According to Ann Arbor 85% (n=22) of patients had advanced disease, stage III-IV, 65% (n=17) with bulky disease, and 35% (n=9) with bone marrow involvement at diagnosis. The IPI score was >2 in 77% (n=20) of patients. There were no statistically significant differences in clinical features at diagnosis between classical BL and Burkitt-like disease. The complete response (CR) rate was 33% and 50% and overall response rate (ORR) 50% and 100% for classical BL and Burkitt-like lymphoma, respectively. After a median follow-up of 22 months (range 6-135) the disease-free survival (DFS) and overall survival (OS) rate was 100% and 50% respectively. The 4-year OS rate was 45% for classical BL and 63% for Burkitt-like lymphoma, respectively ($p=0.11$). Factors predicting for survival were the achievement of CR after four courses of treatment (CR 100%, partial response [PR] 57%, no response/progressive disease [NR/PD] 0%), and staging according to the NCI-staging system for BL. With this system 6 patients resulted to have stage IR-II, 9 IIIA-B, and 11 IVA-C, with 3-year OS rates of 72%, 89%, and 9% respectively ($p \leq 0.0001$). There were no differences in OS between patients receiving IF-RT (n=6), or undergoing surgical resection (n=6) and those who did not, as well as between patients with IPI score < or >2 (67% vs. 45% $p=0.09$). All patients with PD (n=9) died within one year from diagnosis. Of 7 patients with PR, 3 were rescued with salvage therapy (2 high-dose sequential therapy and 1 conventional chemotherapy) and are alive without disease, and 4 died of progressive disease. The treatment was well tolerated and produced low toxicity, with hematologic WHO grade 3-4 toxicity occurring in 82% of cases. Our data show how patients with classical BL or Burkitt-like lymphoma may be effectively treated with the BFM-LNH 81 protocol. The 3-year OS rate for the group of patients with Burkitt-like disease seems to be better than that reported with

regimens for DLBCL. More intensive approaches are needed for patients with advanced disease according to the NCI-staging system and for those not achieving CR after four courses of treatment.

THYROID LYMPHOMA: REPORT OF FOUR CASES WITH CLINICAL AND PATHOLOGIC FEATURES

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Malignant thyroid lymphoma is a very uncommon form of thyroid cancer, accounting for less than 2% of extra nodal lymphomas and only 1-2% of thyroid malignancies, and most commonly occurs in elderly women. Consequently, treatment strategies are based on evidence from a small number of patients.

In this report we describe four cases of NHL of the thyroid gland seen in our center between 1993 and 1999; diagnosis by fine needle biopsy was successfully confirmed by histology.

Four women, age range 70 years, all had a short history of 3-4 months of neck tumor, with a rapidly enlarging mass and symptoms of local compression. In the first case the disease was limited to the thyroid gland and cervical lymph nodes (CS IIEA), in the second case the disease was more advanced with subcutaneous and cutaneous involvement (CS IV EA), with bone marrow infiltration in third case (CS IV B) and diffusion to the lung in the fourth case. Histologic pattern, according to Kiel classification was different: #1 - centroblastic; #2 - immunoblastic; #3 - histiocytic; #4 - large cell B. All four patients were treated with chemotherapy (#1: 6 cycles CVP, #2: 6 cycles PMU+VP16, #3: 6 cycles CHOP, #4: n.6 cycles MiniCEOP). Complete tumor regression was noted in three patients. One patient died owing the heart failure. One patient is in CR one year post chemotherapy. These findings indicate that thyroid lymphoma has a relatively good prognosis.

A review of the literature revealed that most thyroid lymphomas are diffuse histolytic, and treatment and survival rates have been quite variable between institutions.

MEGAKARYOCYTOPOIESIS IN THE PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA IN LONG LASTING REMISSION TREATED WITH ANAGRELIDE

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Anagrelide is a new platelet lowering agent which has been shown to be effective in the control of thrombocytosis in patients with essential thrombocythemia. *In vitro* observations have shown that therapeutic concentrations of anagrelide alter the maturation pattern, size and ploidy of megakaryocytes without inhibiting proliferation (Mazur 1992).

In this study, we investigated the influence of anagrelide treatment *in vivo* on megakaryocyte (MK) size,

number and spontaneous growth in the bone marrow of patients with essential thrombocythemia (ET), successfully treated with anagrelide, with normal number of platelets.

We have analyzed 9 patients with ET who entered to *Open protocol for the use of anagrelide for patients with thrombocythaemia* proposed by Roberts Pharmaceuticals.

The median maintenance dose of anagrelide was 2.5 mg, daily. The median duration of the treatment was 36 months (range 30-60 months). The median platelet count was $370 \times 10^9/L$ (range $240-570 \times 10^9/L$).

In vitro cultures of spontaneous megakaryocyte (CFU-Mk) progenitors were done in the methylcellulose assay.

MK size and number were evaluated by immunomorphometry on trephine biopsies, routinely fixed, paraffin embedded and stained with monoclonal antibody directed against Gp IIIa (CD61, Dakopatts, Denmark), APAAP method.

Our results showed spontaneous CFU-Mk in bone marrow cultures in 3/9 patients with ET who were in stable hematologic response induced by anagrelide.

Mean profile section diameter of MK was significantly higher in ET patients than in controls (25.6 ± 2.9 vs 16.2 ± 1.2 mm, $p=0.00001$) as was MK profile number (76 ± 26 vs. 28 ± 4 MK/mm², $p=0.00001$).

Our results contribute to the preclinical observations that anagrelide does not produce a decrease of platelet counts by altering megakaryocyte progenitor cells, megakaryocyte size or number. According to our results increased number and size of megakaryocytes still persisted in patients with ET in long lasting remission achieved with anagrelide.

IN VITRO CYTOTOXICITY OF FLUDARABINE AND GEMCITABINE ON WIL2-S HUMAN B LYMPHOBLASTOID CELL LINE

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Background and aim. The purine analog fludarabine (F-ara-A) has been demonstrated to be an active treatment of low grade lymphoproliferative disorders; gemcitabine (dFdC) is a new pyrimidine nucleoside analog with established clinical effectiveness against solid tumors, and promising activity in hematologic malignancies (Aleskog et al., *Eur J Haematol* 1999; 62:293-99; Tosi et al., *Haematologica* 1999; 84:794-98). Both drugs inhibit cellular DNA synthesis in the S phase by two different mechanisms: (1) direct termination of DNA strand elongation after the triphosphate metabolites of each drug are incorporated into DNA, and (2) indirect inhibition of DNA synthesis by decreasing cellular deoxynucleotide triphosphates through inhibition of ribonucleotide reductase. Unlike fludarabine, when gemcitabine is incorporated into the elongating DNA strand, the drug residue allows a further addition of a nucleotide by the DNA polymerase, then the synthesis is stopped and the removal of gemcitabine incorporated into the DNA

occurs slowly. In this work, fludarabine and gemcitabine have been examined for their inhibitory effects on cell growth of WIL2-S, a suspension culture of human B lymphoblastoid cells, phenotypically characterized by the expression of the following antigens: HLA-DR⁺, CD5⁻, CD20⁺ and CD95⁺ (Beletskaya et al., *FEBS Lett* 1997; 412:91-3), in order to investigate the relative proportion of their cytotoxic activity and provide the experimental rationale for their possible clinical use in combination regimens for the treatment of lymphoid neoplasms.

Methods. WIL2-S cell line was cultured in RPMI-1640 medium supplemented with 10% fetal calf serum and 1×10^5 cells were plated in 1 mL of medium into each of 24-well plates for cell culture. Graded concentrations of fludarabine (0.01-10 µg/mL) and gemcitabine (0.0001-1 µg/mL) were added to culture wells and each concentration was examined in quadruplicate experiments. Cells were exposed to cytotoxic drugs for 6 hours, then the medium was replaced with drug-free medium and the cells grown for 48 hours at 37 °C in 5% CO₂. At the end of incubation, surviving cells were counted, and the concentration of each drug that produced a 50% inhibition of cell growth as compared to control, drug-free cultures (IC₅₀) was calculated by the CalcuSyn software (Biosoft, UK).

Results and conclusions. The mean IC₅₀ values of fludarabine and gemcitabine on the WIL2-S cell line were 5.1 ng/mL and 0.13 ng/mL, respectively, demonstrating a marked cytotoxic activity of both drugs. If the two chemotherapeutic agents were added simultaneously to cell cultures, a modest enhancement of cytotoxicity was observed. This finding may be explained on the basis of a possible competition of both drugs for the same enzymatic pathway that converts fludarabine and gemcitabine to their cytotoxic metabolites. Therefore, alternative schedules should be explored in order to find the sequence that is associated with synergistic inhibitory effect on cell growth and permits the exploitation of the therapeutic capabilities of fludarabine and gemcitabine in the treatment of lymphoid neoplasms.

LEVELS OF IL-4, IL-10 AND INF-G IN THE SERUM AND IN THE PERIPHERAL BLOOD CULTURE SUPERNATANTS FROM 31 PATIENTS WITH HEMATOLOGIC MALIGNANCIES

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Objectives. We studied the production of TH1-type versus TH2-type cytokines by lymphocytes of patients with hematologic malignancies: these cytokines may play a role in disease progression. Interleukin (IL)-4 and IL-10 are cytokines produced by TH2-type whereas interferon (IFN-) γ is produced by TH1-type lymphocytes. The major antineoplastic activity is played by host's TH1-type cells. A shift from TH1-type cytokines towards TH2-type is considered evidence and a possible cause of cancer progression. **Methods.** We studied the levels of IL-4, IL-10 and IFN- γ in the

serum and in the culture supernatants from PHA- or anti-CD3 monoclonal antibody (MoAb)-stimulated peripheral blood mononuclear cells (PBMC) in 36 patients (mean age 57.3 years, range 28-83) with hematologic malignancies [14 non-Hodgkin's lymphoma (NHL), 6 Hodgkin's lymphoma (HL), 10 multiple myeloma, 4 hairy cell leukemia (HCL), 1 chronic myelogenous leukemia (CML) and 1 chronic lymphocytic leukemia (CLL)]. Ten age-sex-matched normal subjects were used as controls. The patients with NHL, HL and HCL were divided in two groups: those with active disease (AD) and those in clinical complete remission (CR); the patients with myeloma all had AD whereas those with CML and CLL were in CR. **Results.** Serum levels of IL-4 and IFN- γ were in the same range in patients (either AD or CR) and in the control group. Serum levels of IL-10 were significantly higher in patients with NHL, HL and HCL with AD as compared either to patients with NHL, HL and HCL in CR, myeloma, CML, CLL or controls. The levels of IFN- γ in culture supernatants from PHA- or anti-CD3 MoAb-stimulated PBMC were in the same range as in controls and in all patients except that with HCL (AD), in whom the levels were higher. The levels of IL-4 were higher in culture supernatants from PBMC of patients with NHL, HL, HCL (AD) and myeloma than those of patients in CR and controls. The levels of IL-10 were higher in culture supernatants from PBMC of patients with AD than those of patients in CR and controls. The culture supernatants from PBMC of patients with HCL in CR had lower levels of IL-10 than that of the patient with AD, even if those levels were higher than controls. **Conclusions.** These results suggest that in hematologic malignancies, especially with AD, there is a shift from TH1-type to TH2-type cytokine production, which plays a role in disease progression. Only PBMC from a patient with HCL (AD) were able to release high amounts of IFN- γ .

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CLINICAL VALUE OF QUANTITATIVE LONG-TERM ASSESSMENT OF BCR-ABL CHIMERIC TRANSCRIPT IN CHRONIC MYELOGENOUS LEUKEMIA PATIENTS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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For purposes of therapeutic decision making, we used quantitative PCR for molecular follow up of 55 patients in complete remission (CR) from chronic myeloid leukemia (CML) after allogeneic bone marrow transplantation (BMT) from HLA compatible donors. A total of 402 bone marrow samples from 40 patients transplanted in chronic phase (group 1) and 15 in accelerated/blastic phase (group 2) were analyzed by qualitative and quantitative PCR. Regarding clinical outcome, 34/40 (85%) group 1 vs. 8/15

(54%) group 2 patients are alive. Only 1/40 (2.5%) of the group 1 patients relapsed, as against 6/15 (40%) in group 2 ($p = 0.0002$). At qualitative PCR, 8/40 (19%) group 1 vs. 9/15 (60%) group 2 patients were positive, with a significantly greater total number of positive samples in group 2 (33/129, 27% vs. 16/273, 5%; $p < 0.001$). The probability of qualitative PCR positivity >1 year after BMT was significantly lower in group 1 patients (4/40 patients, 10% vs. 9/15 pts, 60%; $p = 0.01$). At quantitative PCR, 4/8 (50%) group 1 patients were positive only once (< 400 transcripts/ μ g RNA). In group 2, 9/15 (60%) patients had 3 or more positive samples (always with >4,000 copies/ μ g RNA); therapeutic interventions (cyclosporin A discontinuation, temporary α -interferon or donor lymphocyte infusion) restored molecular remission in 4/9 (44%) cases.

This study indicates that quantitative PCR could provide practical indications capable of directing therapeutic interventions for transplanted CML patients, especially those transplanted in accelerated/blastic phase, for whom intensive monitoring is required.

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EFFECTS OF TOPICAL APPLICATION OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR ON ORAL MUCOSITIS IN MYELOMA PATIENTS TREATED WITH INTENSIFIED CHEMOTHERAPY

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Oral mucositis is a serious, almost inevitable complication in patients with neoplastic diseases treated with high dose chemotherapy. It further debilitates patients by provoking dysphagia, weight loss, need of parenteral nutrition and facilitates the possibility of secondary local and systemic infections. Subcutaneous administration of granulocyte macrophage colony-stimulating factor (GM-CSF) has beneficial effects in reducing the severity and duration of oral mucositis in chemo- and radiotherapy-treated patients. More recently, local administration of GM-CSF has shown to be effective in wound healing and in reducing radiation-induced oral mucositis in cancer patients. The mechanism of action of GM-CSF in this setting is still not clear. Direct or cytokine-mediated stimulation of growth and functions of fibroblasts, myofibroblasts, endothelial cells, Langerhans cells and keratinocytes, as well as a contribution to primary immune responses against various pathogens, have been suggested.

We evaluated the effect of locally administered GM-CSF on oral mucositis in fifteen 60-to-70 year-old patients with multiple myeloma (MM), who underwent two courses of "intensified" chemotherapy, each with i.v. melphalan (100 mg/m²) followed by infu-

sion of previously collected autologous CD34⁺ peripheral blood progenitor cells (PBPC). An interval of two months separated the cycles. All patients received standard oral prophylaxis with ciprofloxacin and fluconazole and subcutaneous G-CSF until WBC recovery. After the second PBPC infusion, oral GM-CSF was given to all patients, so that every subject could be considered as his own control. Oral GM-CSF solution was obtained by diluting 300 µg of standard GM-CSF preparation (Mielogen, Schering-Plough) which was added to a glass containing about 200 mL of drinking water. The patients were instructed to use this solution as a mouthwash and then swallow it, in fragments, within 1 hour. Oral mucositis was graded from 0 to 4, according to a previously reported score based on gross (by physician: erythema, ulcers, pseudomembranes, necrosis and hemorrhages) and functional (by patient: soreness, local pain, dysphagia, type of diet, parenteral support) evaluations. The comparison between the two courses of intensified chemotherapy showed that, despite the fact that the period with neutrophils < 500/µL was about one day longer after the second *mini-transplant* (median 5.8 vs 4.7 days), both the duration (median 4.5 vs 7 days, $p < 0.04$) and the severity (median score 1.1 vs 2.2, $p < 0.04$) of oral mucositis were significantly reduced when oral GM-CSF was administered. This study suggest that local GM-CSF may be useful in reducing oral mucositis in myeloma patients treated with intensified chemotherapy. The role of this approach in more intensively treated patients warrants evaluation.

ERYTHROPOIETIN IN CONJUNCTION WITH HIGH DOSE VP16 AND LENOGRASIM ENABLES PROGENITOR CELL COLLECTION IN MULTIPLE MYELOMA PATIENTS WHO FAILED CYCLOPHOSPHAMIDE INDUCED MOBILIZATION

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Currently high dose cyclophosphamide (CTX) 4-7 g/m² plus hematopoietic growth factors is considered one of the most effective methods to mobilize peripheral blood progenitor cell (PBPC) even though in multiple myeloma (MM) patients, PBPC harvesting might be more difficult than in patients with other malignancies. Recent observations *in vivo* suggested that erythropoietin, when used in addition to chemotherapy and G-CSF, enhances PBPC mobilization and that high dose VP16 or VP16-containing regimen may be considered suitable alternatives for those patients who fail CTX induced mobilization.

We report our experience with a high dose of etoposide (VP16) 2 g/m² plus lenograstim (Myelostim) 10 µg/kg started 24 h after chemotherapy and administered until the last day of leukapheresis plus erythropoietin (rhEPO-Eprex) 150 IU/kg every other day from day 2 to day 13, in five patients with MM who failed to mobilize PBPC with CTX 5 g/m² plus G-CSF 10 µg/kg (CD34⁺ cells/µL median peak values: 8.5) after 3 regimens of VAD and before melphalan 100 mg/m² (Mei 100) at days 30; 90; and 150. All patients had

stage III disease (Durie & Salmon). Their mean age was 55 years; PS 1-2. There were 2 female, and 3 males. Three had IgGκ, 1 IgGλ, and 1 IgAκ. VP16 plus lenograstim and erythropoietin resulted in a statistically significant ($p < 0.5$) increase of day 11 and 12 incidence of CD34⁺ cells/µL median peak values 105.5 (vs 8.5). In conclusion, our results suggest that VP16 plus lenograstim and erythropoietin is a highly effective mobilization regimen for those patients who fail CTX induced mobilization.

PERFORIN-EXPRESSING CIRCULATING CD4⁺ CELLS IN PATIENTS WITH B CHRONIC LYMPHOCYTIC LEUKEMIA PREDOMINANTLY HAVE A CYTOTOXIC AND ACTIVATED PHENOTYPE

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Introduction. The role of T cytotoxicity in B-cell chronic lymphocytic leukemia (B-CLL) remains unclear. Allogeneic CD8⁺ T cells can be activated to acquire cytotoxicity and the few studies focused on cytotoxic CD4⁺ T cells in this disease have shown the non-specific cytotoxic function of CD4⁺ cells to be limited to the CD57⁺ phenotype. We have recently shown an increase in B-CLL of potentially cytotoxic CD4⁺T cells by their expression of perforin (PF) and serine esterase.^{1,2}

Methods. In order to define the phenotype and activation status of PF-expressing T cells in B-CLL we used three-color immunofluorescence. Peripheral blood lymphocytes from 14 Rai staged untreated B-CLL patients and 10 age-matched controls were stained for surface expression of CD4 or CD8 with CyC-conjugated monoclonal antibodies (mAbs), and for an additional marker CD28, CD69, HLA-DR, CD57, CD45RO and CD45RA with PE-conjugated mAbs. The cells were then fixed, permeabilized, stained for intracellular PF using FITC-conjugated anti-PF mAb and analyzed by flow cytometry.

Results. Eleven of the 14 B-CLL patients had higher percentages ($p < 0.001$) of circulating CD4⁺PF⁺ cells (mean 14.1±11.6%, range 1.4-42.9%) than controls (mean 2.0±1.7%, range 0.1-4.8%). The expression of PF by CD4⁺ cells was confirmed by confocal microscopy. Preliminary data suggest that it was augmented at later stages of the disease, compared with stage 0. There was also a significant ($p < 0.001$) increase in PF producing CD8⁺ cells (mean 55.6±17.3%, range 26.7-82.3%), compared with healthy controls (mean 28.3±16.3%, range 2.4-61.9%). We found that both, CD4⁺ and CD8⁺ PF-producing T cell subsets are enriched with CD57⁺ and CD28⁻ cells (see Table). This is consistent with them having a cytolytic function. PF-expressing CD4⁺ cells were predominantly of the CD45RO activated/memory cell phenotype ($p < 0.01$), while the PF⁺ and PF⁻ CD8⁺ cell populations contained some cells expressing both CD45RO and CD45RA cells. Although PF⁺CD4⁺ cells had low expression of the early activation marker

CD69, they were enriched with the later activation CD28⁻ phenotype. The decreased number of HLA-DR⁺ cells among PF⁻ and PF⁺CD4⁺ subsets might reflect an aberrant expression of HLA class II antigens on these cells.

Conclusions. The phenotype of perforin-expressing CD4⁺ cells suggests their activated status and potential cytotoxic function.

Table. Phenotype of PF-expressing CD4⁺ and CD8⁺ cells in B-CLL.

Cell subset	CD57	CD28	CD45RO	CD45RA	CD69	HLA-DR
PF-CD4 ⁺	55.3±7.4*	18.9±7.3*	77.7±10.2	21.2±8.3	3.8±1.9	6.9±2.5
PF-CD4 ⁺	14.6±4.0	77.1±5.5	66.1±10.2	32.9±6.9	4.0±1.8	6.2±2.2
PF-CD8 ⁺	83.3±3.8†	16.4±12.0†	56.2±12.3	64.5±14.8	8.4±2.5	25.6±8.7
PF-CD8 ⁺	42.1±7.8	43.9±8.8	55.8±9.4	55.7±15.6	7.1±2.2	20.0±9.9

Mann-Whitney test, mean±standard error, **p*<0.001; †*p*<0.01; ‡*p*<0.05, CD4⁺ and CD8⁺ PF⁺ cells compared with the corresponding PF⁻ subset.

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PAMIDRONATE AFFECTS GAMMAGLOBULIN SECRETION AND BONE DENSITY IN PLASMACELL DYSCRASIAS

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In vitro and *in vivo* studies have suggested that pamidronate possesses antiproliferative activity on neoplastic plasmacells. Thus, in addition to the inhibitory action on bone resorption, bisphosphonates may exert positive effects on plasmacell dyscrasia by interfering with the basic cellular processes implicated in these pathologic states. To determine whether immunoglobulin (Ig) secretion may be affected by bisphosphonates, 20 patients with MGUS, 10 with smoldering myeloma (SM), 10 with multiple myeloma (MM) and 2 with Waldenström macroglobulinemia were treated with monthly or biweekly cycles of intravenous 90 mg pamidronate. Qualitative and quantitative characterization of bone marrow plasmacells were obtained in each patient by histology and immunohistochemical detection of monoclonal light chain expression. The serum monoclonal secretion was measured by electrophoresis, immunofixation and nephelometry. Hematologic and biochemical parameters were monitored throughout the course of therapy. In addition, bone mineral density (BMD) was evaluated at the beginning and at the end of treatment. Results indicate that pamidronate significantly decreased by 20%

(*p*<0.05) (range: 3-48%) the serum globulin levels in MGUS, was effective in the control of paraproteinemic values in SM, whereas the M component did not statistically vary in MM within the time of observation. No changes were observed in peripheral blood counts whereas serum alkaline phosphatase was reduced by 30% (*p*<0.005). After pamidronate administration amelioration of BMD was obtained in only a fraction of MGUS, SM and MM. The clinical and laboratory findings of the short term follow-up of the entire population was also evaluated to detect significant relationships with treatment. No major toxicity was observed and 90% of patients had a clinical benefit.

In conclusion, pamidronate affects the secretory capacity of plasmacells suggesting its potential role in the control of neoplastic and non-neoplastic plasmacell disorders.

IN VITRO INHIBITION OF LECTIN-STIMULATED AGGREGATION OF LEUKOCYTES IN PLATELET-RICH PLASMA BY ANTITHROMBIN-III CONCENTRATE

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Introduction. Glycosaminoglycan-mediated aggregation of cells occurs through a heterophilic adhesion mechanism in which heparan sulfate chains bind to a counter-receptor on these cells. As antithrombin-III (AT-III) interacts with heparan sulfate proteoglycans through its heparin-binding domain and inhibits leukocyte adhesion in ischemia/reperfusion and sepsis models, it may affect leukocyte aggregation.

Methods. Leukocyte (0.65×10^7 /mL) aggregation was monitored as the increase in transmission of light through stirred suspensions in a platelet aggregometer (Born/Michal MK IV). Aggregation curves were quantified as the area under the curve in the first 6 minutes following stimulation. Leukocytes in platelet-rich plasma (LPRP) were obtained from heparinized whole blood of healthy donors by centrifugation; the ratio of leukocytes to platelets was about 1/50, and the final concentration of autologous plasma was 80%. Neutrophils (PMN) were isolated by dextran sedimentation, density centrifugation and hypotonic lysis of erythrocytes. Aggregation was induced by phytohemagglutinin (PHA; 0.24 mg/mL) or formyl-Met-Leu-Phe (FMLP; 0.2×10^{-6} M), with or without various concentrations of AT-III (Kyberlin[®], Aventis Behring).

Results. During the observation period (6 min) no aggregation of LPRP or isolated PMN could be induced either with medium or with AT-III (0.2×10^3 to 0.2×10^6 U/mL). Addition of PHA or FMLP with different potencies stimulated aggregation of LPRP and of PMN. Additional presence of AT-III significantly inhibited PHA-induced aggregation of LPRP, whereas FMLP-induced aggregation was not affected by AT-III. In contrast, PHA-induced homotypic aggregation of PMN was augmented by AT-III when no plasma (thrombin) a platelets were present. FMLP-induced

aggregation of PMN was again, not affected by AT-III.

Conclusions. In the presence of plasma and platelets, aggregation of normal white blood cells after stimulation with PHA but not with FMLP can be inhibited by AT-III concentrate suggesting a lectin receptor-dependent mechanism in immunomodulatory effects of the serpin. As AT-III augments PHA-induced aggregation of isolated PMN, net effects of AT-III appear to be dependent on additional blood elements.

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DIFFUSE LARGE CELL LYMPHOMA IN ELDERLY PATIENTS TREATED WITH MiCEP PROTOCOL.

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Cancer is predominantly a disease of the elderly. Chemotherapy is one of the primary options in the treatment of cancer but chemotherapy does present problems in older patients. The increasing incidence of non-Hodgkin's lymphoma (NHL) in elderly people led to specific regimens being designed for these patients in a pathology particularly responsive to this type of treatment. Since 1989 in order to reduce organ toxic-

ity we devised a novel chemotherapy scheme utilizing drugs with limited organ-specific toxicity: the MiCEP regimen-mitoxantrone 9 mg/m² day 1; cyclophosphamide 300 mg/m² on days 2, 3 and 10, 11; etoposide 50 mg/m² on days 1, 2 and 9, 10 and prednisone 40 mg/m² from day 1 to day 11. We presented the preliminary results of this protocol on Leukemia and Lymphoma in 1995. Up to 1996 we had collected 145 patients older than 65 years with a *de novo* diagnosis of high grade NHL. These patients were treated with the MiCEP protocol between 1989 and 1996. Their median age was 72.3 years (range 65-87); 78 patients (54%) were in stage III-IV, 40 (27.5%) were symptomatic, 38 (26%) had an LDH value higher than normal and 18 (12%) had bulky disease. According to IPI score 48 (33%) were low-risk, 40 (28%) were low-intermediate risk, 39 (27%) were intermediate-high risk and 18 (12%) were high risk. Sixty-three percent (91/145) achieved a complete remission (CR), 48 (33%) obtained a partial response and 6 were non responders. The overall response rate was 96%. With a median follow-up of 55 months (range 1-138 months) overall survival and progression-free survival at 5 years were, respectively, 52% and 43%. In a multivariate analysis response to therapy was the only one parameter statistically significant for overall survival. Twenty-four out 91 CR patients (26%) relapsed, the majority in the first year of treatment and the disease-free survival for CRs was 68% with a median follow-up of 49 months (range 2-116 months). The treatment was well tolerated; we recorded five early deaths due to complication of therapy (3%). In conclusion our data confirm the utility of regimens specifically devised for elderly patients to reduce toxicity and to obtain a high rate of CR which is the single parameter significantly related to overall survival.