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38° Congress of the Italian Society of Hematology
Florence, October 7-10, 2001
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The Scientific Committee, The President and the Vice Presidents of the 38th Congress of the Italian Society of Hematology would like to thank the 66 Reviewers for their strong co-operation in evaluating and selecting the 635 received abstracts.

Each abstract has been independently assessed by 3 different reviewers according to a score system based on the parameters of originality, methodological approach and general interest. Sixteen abstracts with the highest score will be presented in the plenary session as selected communications. A further 150 abstracts have been chosen for oral presentation and finally 419 will be presented as posters.

Special thanks to the Management Committee of "Haematologica" for the co-operation in the diffusion of the relevant advances in hematology presented at the 38th Congress of the Italian Society of Hematology.

Pierluigi Rossi Ferrini
Florence, October 2001
We have recently studied an autosomal dominant macrothrombocytopenia characterized by mild or no clinical symptoms, normal platelet function activity, and normal megakaryocyte count. In an attempt to identify the molecular basis of the disease, linkage analysis in two large families localized the gene to chromosome 17p, in an interval containing the GPIbα gene, which is altered in Bernard-Soulier syndrome (BSS). A heterozygous Ala156Val missense substitution (Bolzano variant) was identified in all patients of the two families and in another six additional macrothrombocytopenic pedigrees. BBS is an autosomal recessive disorder characterized by prolonged bleeding time, thrombocytopenia, and large platelets. The molecular basis of the disease is due to a defect of the platelet glycoprotein (GP) Ib/IX/V complex, which is the platelet receptor for plasma von Willebrand factor (vWF) and the major membrane GP system. GPIb/IX/V consists of four distinct gene products, GPIbα, GPIbβ, GPIX, and GPV. BBS patients are homozygotes or compound heterozygotes for mutations in the GPIbα, GPIbβ, or GPIX genes. Consistent with a BSS heterozygous condition, the vWF receptor GPs were reduced in all patients with the Bolzano variant found in this study. Thus, the diagnosis of heterozygous BSS must always be suspected in those individuals with inherited thrombocytopenia and platelet macrocytosis. Platelet membrane GP studies were also performed on families characterized by macrothrombocytopenia without the Bolzano variant. The analysis distinguished two groups: 1) patients (63) with the GPIb/IX/V complex normally distributed on the surface of their platelets. We called this form true autosomal dominant macrothrombocytopenia; 2) patients (6) with a reduction of GPs comparable to that found in the BSS heterozygotes. We hypothesized that mutations in the BSS gene were responsible for the phenotype of the second group. Thus, the coding region of GPIbα, GPIbβ, and GPIX, as well as GPV, including the intronic flanking sequences and the promoter region, were amplified in one proband from each family. The presence of the fragments of the predicted size did not reveal gross alterations and the automated sequencing analysis of the PCR product excluded the presence of mutations. These results suggest that there is at least another gene, not assembled in the GPIb/IX/V complex, responsible for the BSS heterozygous phenotype. We can also speculate that there might be patients, affected by the same severe symptoms as in the recessive BSS, carrying mutations in both the alleles of this putative gene. A positional cloning strategy based on linkage analysis and mutation screening in candidates is in progress to identify the gene.
CS003
PEG-INTERFERON α-2b IN ESSENTIAL THROMBOCYTHEMIA: PHASE II STUDY FOR DETERMINATION OF THE MINIMUM EFFECTIVE, SAFE AND TOLERATED DOSE. PRELIMINARY DATA


Hematologic Departments of Ancona, Bergamo, Bologna, Castelfranco Veneto, Catania, Firenze, Genova, Modena, Monza, Napoli, Pavia, Perugia, Ravenna, Reggio Emilia, Roma, Siena, Taranto, Torino, Udine

Interferons (IFN) α, β or γ (Interleukin-2, IL-2) are biologic modifiers with antiproliferative and antiviral effects. In contrast to other antineoplastic drugs they are relatively nontoxic and have a number of advantages: a) short half-life; b) nonspecific activity; c) low cumulative toxicity; d) differentiation inducing activity; e) ability to induce regulatory T lymphocytes. The basis of IFN-α activity is believed to be a combination of direct antiproliferative effects and immunomodulatory effects. The latter include the activation of the immune system through the enhancement of T and NK cell function and the enhancement of the expression of adhesion molecules, which results in their increased binding to target cells. The antiproliferative effect is probably mediated by the induction of the expression of IFN-inducible proteins that block the cell cycle at specific checkpoints. This effect is enhanced by the prolongation of the half-life of the drug by pegylation.

CS004
STI571 IN THE TREATMENT OF PH+ CHRONIC MYELOID LEUKEMIA, IN ACCELERATED AND BLASTIC PHASE: THE PRELIMINARY EXPERIENCE OF THE ITALIAN COOPERATIVE STUDY GROUP ON CML (PROTOCOL CML/003/STI571)


Institute of Hematology and Medical Oncology "L. e A. Seràgnoli"*, Ospedale S.Orsola, Bologna University; *Chair of Hematology, Udine University; Novartis Farma, Origgio (VA); §Chair of Medical Pathology, Orbassano (Torino)

The Italian Cooperative Study Group on CML between August, 2000 and May 2001 has completed the recruitment time of a phase II, multicenter, observational study, aimed to evaluate the efficacy and safety of a inhibitor of the protein-tyrosine kinase Bcr-Abl associated, STI571, in adult patients with Ph+ chronic myeloid leukemia in accelerated and blastic phase. The primary end-point of this study, of 6 months of duration, was the rate of hematologic response. Moreover, among secondary objectives, the study scheduled the prospective evaluation of the molecular response to STI571, the serial, quantitative analysis of bone marrow and peripheral blood samples taken at fixed time points (4 in 6 months). Patients received STI571, 600 mg once a day, orally, except when adverse events required a dose reduction/interruption, for at least 6 months. A dose escalation to 800 mg/daily (400 mg/12 hours) was scheduled for patients further resistant or refractory to STI571 overall. 45 Italian hematologic institutions belonging to the group enrolled 215 patients between August 17th, 2000 and May 10th, 2001. The preliminary results of this study will be presented.

CS005
CHARACTERIZATION OF A NEW SURFACE RECEPTOR (IRTA-1) EXPRESSED ON NORMAL AND NEOPLASTICAL MARGINAL ZONE B CELLS

B. Falini,* E. Tiacci†, B. Bigerna,* A. Pucciarini*, L. Pasqualucci,** R. Kuppers,** R. Dalla Favera**

*Institute of Hematology, University of Perugia; **Institute of Pathology, Columbia University, New York

The high frequency of genetical alterations involving the band 1q21 in human B cell lymphomas suggests that this locus may contain genes playing a key role in normal B cell ontogenesis and lymphomagenesis. Recently, the breakpoint corresponding to the rare chromosomal translocation (1;14)(q21;q32) of the human myeloma cell line RPMI8226 has been cloned, leading to the identification of a new gene named IRA-I (Immunoglobulin Superfamily Receptor Tralocation associated-1 gene) that encodes for the corresponding immune receptor. The IRA-I molecule shares homologies with the members of the Fc receptor family and the Inhibitory Receptor Superfamily (IRS), and with the adhesion molecules of the CAM family. This finding suggest that the IRA-I immune receptor may be involved in signal transduction during immune response (as the Fc receptors), in the intercellular communication (as the
members of the IRS family) and in the mechanism of cell adhesion (as the members of the CAM family). Recently, we have generated a monoclonal antibody (named MIRTA-1) directed against a fixative-resistant epitope of the IRTA-1 molecule. This antibody was used to investigate by immunohistochemistry the expression of IRTA-1 in normal and pathological lymphoid tissues. In normal lympho-hemopoietic tissues, the IRTA-1 molecule appears to be mainly expressed on lymphoid elements selectively distributed in areas corresponding to the so-called marginal zone. The B cell nature of such IRTA-1 positive cells have been confirmed by double immuno-enzymatic stainings and by single cell PCR studies performed on IRTA-1 positive which have been cell picked-up with a mechanical micromanipulator from frozen sections of normal human tonsil. The single cell PCR analysis has demonstrated that the IRTA-1 positive cells represent a genotypically heterogeneous population, as far as concerns mutations of the immunoglobulin genes. In the context of B-cell lymphomas, expression of IRTA-1 has been mostly detected in nodal and extranodal non-Hodgkin B-cell lymphomas of the marginal zone. In particular, the molecule appears to be strongly expressed on the surface of tumor cells that participate to the formation of the so-called lympho-epithelial lesions. The monoclonal antibody MIRTA-1 represents the first marker of marginal zone B cells and it is expected to be a valuable reagent for the study of normal B cell ontogenesis and the characterization of human lymphomas.

CS006
BONE MARROW MESENCHYMAL STEM CELLS COUNTERACT SUPPRESSIVE EFFECTS INDUCED BY ONCOSTATIN M ON FETAL HEMATOPOIETIC PROGENITORS EXPANDED IN LIQUID PHASE CULTURES BY “EARLY ACTING” CYTOKINES

L. Luchetti, A. Gallaszzi, L. Forte, C. Romaniini,* G. De Rossi,* G. Isacchi,* A. Tocci
Stem Cell Laboratory & **Department of Hematology and Immunotransfusion Medicine, Bambino Gesù Pediatric Hospital and *Department of Gynecology and Obstetrics, Tor Vergata University, Rome

Mesenchymal stem cells (MSC) were recently isolated from human bone marrow stroma. The role of MSC in sustaining hematopoiesis and proliferation of hematopoietic cells was suggested but not explained. Oncostatin M (OSM), which belongs to the gp130 family of cytokines, and is expressed in the aorta-gonad-mesonephros region and murine fetuses, stimulates the maturation of hepatic parenchymal cells and terminates embryonic liver hematopoiesis. However, its role in hematopoiesis and interaction with the medullary microenvironment remain unexplained in humans. Furthermore, human MSC are reported as not expressing OSM. This study investigates the role played by MSC in the expansion of fetal (cord blood) hematopoietic progenitor cells (HPC) mediated by early acting cytokines and the effect of adding OSM. CD34+ cells were purified (Miltenyi) (phenotypic purity 97±2.1%, blasts>98%, N=4). MSC were obtained in FCS+ cultures (HPC) mediated by embryo extract, VEGF or α-MSH. CD34+ cells were cultured for 7 days in the presence of FCS, early acting cytokines SCF, FLT3, TPO (SCF/FLT/TPO) and/or MSC irradiated (80Gy) to halt proliferation. The effect of adding OSM was also investigated. The nucleated cell number (NC), the phenotype (CD34 and CD45 expression), the morphology (May-Grünwald-Giemsa) and the clonogenic potential (HPC in semisolid cultures supplemented with SCF/IL-3/GM-CSF/Epo) were evaluated. It was observed that: (i) NC expand in the presence of SCF/FLT/TPO, while adding OSM reduces expansion. The presence of MSC does not significantly modify the number of expanded NC, although it counteracts the inhibitory effect induced by OSM; (ii) CD34+ cell frequency, analyzed by FACS in the population expanded by SCF/FLT/TPO, does not change at 20% and is not modified by MSC, with or without adding OSM; (iii) immature myeloblastic phenotype is represented in 54% cells expanded with SCF/FLT/TPO, but rises to 92% in the presence of MSC. Adding OSM to MSC and SCF/FLT/TPO does not modify the frequency of immature myeloblasts (90%). However, most cells display very prominent nucleoli, unless only SCF/FLT/TPO and MSC are present; (iv) the presence of MSC favors the expansion of HPC induced by SCF/FLT/TPO (32 vs 12 fold, presence vs absence of MSC, respectively). The effect is particularly evident with mixed HPC (CFU-GEM M) (37 vs 7 fold); (v) in the absence of MSC, OSM counteracts the expansion induced by SCF/FLT/TPO (2 vs 12 fold); (vi) MSC were found to abolish the inhibitory effect of OSM on the expansion induced by SCF/FLT/TPO (2 vs 31 fold in, respectively, the absence or presence of MSC). This study demonstrates that: (i) MSC maintain fetal CD34+ cells; (ii) MSC promote the expansion of HPC induced by early acting cytokines; (iii) OSM counteracts the expansion of HPC induced by early acting cytokines, in the absence of MSC; (iv) MSC restore the capability of SCF/FLT/TPO to expand fetal progenitors, in the presence of OSM. This study suggests a modulatory role of MSC in the transition of human fetal hematopoiesis, by inhibiting, at medullary level, the suppressive effects of OSM exerted in fetal liver.

CS007
IN VITRO AND IN VIVO EVALUATION OF THE HEMATOPOIETIC POTENTIAL OF HUMAN MUSCLE CELLS

Hematology-Oncology, Experimental Oncology and Pathology, European Institute of Oncology, Milan

It has been recently demonstrated in the mouse model that the potential of stem cells is not restricted by their source. In mice, brain cells can reconstitute hematopoiesis, and bone marrow (BM) cells can generate muscle, brain, endothelial and liver tissue. Jackson et al. (PNAS 1999) demonstrated that murine muscle cells can reconstitute hematopoiesis in lethally irradiated mice. We studied the engraftment potential of human fresh and cultured cells generated from muscle surgically removed from head and neck cancer patients. Muscle samples were minced, digested and cultured in F12-10% FBS. NOD/SCID mice (n=25) were transplanted with fresh or cultured human muscle cells. Compared to mice, in vitro proliferation of human muscle cells was slower and not increased by the addition of chick embryo extract, VEGF or IGF-I, or co-culture with human stromal cell lines expressing SCF, G-CSF, GM-CSF, IL-1β, IL-6, IL-7, IL-8, IL-11, LIF, M-CSF, MIP-1α, TGFβ, and TNFα. On day 12 we obtained a median of 500,000 adherent cells/g of muscle sam-
ple. Cultured human muscle cells were evaluated by flow cytometry for the expression of a panel of hematopoietic, endothelial, stromal and epithelial markers. At the beginning and at the end of muscle cell culture, contamination by hematopoietic (CD45+) and endothelial (CD31+) cells was always <1%. On day 12 of culture, CD34 (Thy-1), HER2/neu and PI3K were expressed in most cells. CD34, c-kit, and CXCR4 were expressed by 0.5-1.5%, 1-15%, and 30% of cells, respectively. CD133 and CD135 (flt-3) were not expressed, and KDR appeared to be downregulated. In methylcellulose, fresh (but not cultured) muscle cells generated CFU-GM and BFU-E. NOD/SCID mice transplanted with fresh muscle cells or less than 500,000 cultured cells showed little or no human engraftment. In mice transplanted with more than 500,000 cultured cells, up to 14% human CD45+ hematopoietic cells (including myeloid and lymphoid subsets) were detected by flow cytometry in the BM and peripheral blood. Results were confirmed by PCR, Southern blotting and DNA sequencing. Liver, muscle and spleen evaluated for human DNA were positive in the large majority of mice transplanted with cultured cells. Taken together, our data indicate that human muscle cells generate multilineage hematopoiesis in NOD/SCID mice. Surprisingly, this hematopoietic potential increases in cultured vs fresh muscle cells. Thus, muscle cells are particularly attractive for ex vivo expansion and retrovirus-mediated gene transfer. We are evaluating the engraftment potential of different subsets of muscle cells, and whether muscle cells may generate hematocytes in mice recipients.

CS008
THE GATA-1 LOW MICE: A NEW ANIMAL MODEL FOR THE PATHOGENESIS OF MYELOFIBROSIS IN HUMANS


Idiopathic myelofibrosis is a rare clonal myeloproliferative disorder of unknown etiopathology whose hallmark is extensive marrow fibrosis accompanied by extramedullary hematopoiesis. The finding that mice over-expressing thrombopoietin, or its receptor Mpl, developed myelofibrosis suggested a link between megakaryocyte accumulation and development of the disease. In fact, mice lacking the first Gata-1 enhancer and its distal promoter (knockdown mutants, Gata-1 low mice) are unable to express GATA-1 in megakaryocytes, which fail to differentiate thus leading to a severe thrombocytopenia; on the other hand, GATA-1 expression in erythroid cells is partially maintained, although at low levels (by flow cytometry in the BM and peripheral blood). Results were confirmed by PCR, Southern blotting and DNA sequencing. Liver, muscle and spleen evaluated for human DNA were positive in the large majority of mice transplanted with cultured cells. Taken together, our data indicate that human muscle cells generate multilineage hematopoiesis in NOD/SCID mice. Surprisingly, this hematopoietic potential increases in cultured vs fresh muscle cells. Thus, muscle cells are particularly attractive for ex vivo expansion and retrovirus-mediated gene transfer. We are evaluating the engraftment potential of different subsets of muscle cells, and whether muscle cells may generate hematocytes in mice recipients.

CS009
INCIDENCE AND MOLECULAR EPIDEMIOLOGY OF PSEUDOMONAS AERUGINOSA INFECTIONS IN ACUTE LEUKEMIC PATIENTS

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The incidence and molecular epidemiology of Pseudomonas aeruginosa bacteraemias were monitored in acute leukemic patients over a 3-year period to define mechanisms of possible nosocomial transmission. From September 1996 to December 1999, 309 febrile episodes occurred in 187 patients; of 139 organisms isolated in 116 bacteraemias (14 were polymicrobial), 67 (48%) were gram negative bacilli (GNB). Among the GNB sepsis, we recorded 34 (51%) P. aeruginosa bacteraemias (27 monomicrobial, 7 polymicrobial): 3 in 1996, 10 in 1997, 11 in 1998, 10 in 1999. All patients, who were allocated multiple bedded rooms, had severe neutropenia (PMN < 100/m3) and received a potent antipseudomonal coverage. Of these, 13 died within a few days of onset (9 in monomicrobial sepsis). Patients with P. aeruginosa infections are isolated and rooms are regularly cleaned and disinfected. All 34 clinical isolates were studied with pulsed-field gel electrophoresis (PFGE). According to Tenover (1995), strains were judged to be related if the pattern was either identical or differed by fewer than seven bands (closely related with 1-3 band differences, possibly related with 4-6 band differences) and unrelated if band differences were seven or more. Evaluation of DNA correlation showed: 2 related in 1996, 7 related in 1997, 10 related in 1998, 7 related in 1999 (mainly closely and possibly related). Isolates closely related between themselves were also possibly related with other strains. About 60% of patients with related strains were hospitalized in the same room or in different rooms, but became infected in the same period. The high correlation among clinical isolates, suggests a horizontal spread among the patients, even if other sources, such as hospital personnel via hands, contaminated bio materials or the inanimate environment as well as sanitary facilities may be the most likely route for exogenous nosocomial acquisition of strains. Preliminary epidemiologic environmental search revealed P. aeruginosa in several sanitary services and...
further control measures to improve environmental disinfection are in progress. This study assessed the usefulness of typing analysis in bacteriological epidemiology.

CS010
TRANSFORMING GROWTH FACTOR-β1 CAUSES TRANSCRIPTIONAL ACTIVATION OF CD34 AND STAGE-SPECIFIC EFFECTS ON STEM CELL DIFFERENTIATION

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Human CD34+ stem/progenitor cells have in vitro and in vivo hematopoietic activity and, likely, originate from CD34+ stem cells. TGF-β1 is one of the soluble molecules which regulate cell cycle and differentiation of hematopoietic cells, but has pleiotropic activities depending on the state of responsiveness of the target cells. In this work, we show that TGF-β1 upregulates the human CD34 antigen, an effect visible both at transcriptional and protein level. This effect was striking in primary progenitor cells (CD34-Lin- and CD34+Lin-) and in the TF-1 cell line. CD34 modulation by TGF-β1 did not affect cell growth but influenced differentiation in a stage-specific manner, promoting differentiation of CD34+Lin- cells while maintaining primarily CD34+Lin- in an undifferentiated state. This effect was associated with Smad protein activation and with a dramatic decrease in p38 phosphorylation in CD34+Lin- cells. Conversely, no specific effects on Smad or p38 proteins were observed in CD34-Lin- after exposure to TGF-β1. Moreover, block of p38 phosphorylation by SB202190 inhibitor did not enhance CD34 expression in CD34+Lin- cells, confirming that the p38 pathway is not upstream. CD34 gene transcription and CD34 regulation by TGF-β1 probably occur through unknown transduction signals. These data establish the role that TGF-β1 has in the modulation of the CD34 antigen, providing important clues for understanding hematopoietic development and a potential tool for the modulation of stem/progenitor functions.

CS011
ANTI-FUNGAL PROPHYLAXIS IN MISMATCHED TRANSPLANTS

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Incompatible hematopoietic stem cell transplants are performed at our center for high-risk leukemia patients lacking a matched donor (Aversa et al. NEJM 1998; 339:1186). Fungal infections remain a major obstacle for successful outcome in these patients even in absence of neutropenia and GvHD. Risk factors include deficit in T-cell recovery, advanced stage of disease and the use of immunosuppressive agents including G-CSF (Volpi et al. Blood 2001; 97:2514). Unlike other type of transplanted patients, they all need to receive prophylaxis. As conventional amphotericin (CA) was not suitable for long-term anti-fungal prophylaxis because of its side effects in these patients, we opted for ambisome. In order to investigate efficacy and toxicity of liposomal amphotericin (LA) (ambisome) in preventing fungal infections, a protocol for fungal prophylaxis was tested in 143 high-risk leukemia patients transplanted between October 1995 and October 2000. Results were compared with those of a previous series of 36 high-risk leukemia patients who had received either fluconazole or CA. Median age of the 143 patients was 32 (range 4-62) years, diagnoses included 59 AML, 38 ALL and 46 CML. Almost all were at high-risk for transplant-related mortality and relapse (80 in relapse, 47 in CR=II and 16 in bad-risk CR I). Anti-fungal prophylaxis consisted of ambisome at 1 mg/kg from day -10 to day +30 and then according to risk factors, of either ambisome (3 mg/kg × 3 weekly) or itraconazole for up to 4 months. Conditioning consisted of TBI in a single faction, thiopeta, rabbit ATG and fludarabine. Patients received a median of 10 × 10^6 CD34+ cells/kg and 3 × 10^6 CD34+ cells/kg. No post-transplant immunosuppressive therapy was given as GvHD prophylaxis. Overall 140 (99%) patients achieved a full donor-type engraftment (10 after a second transplant). Only 13 patients developed GvHD. At a median follow-up of 24 (range 8-65) months, 32 patients survive disease-free. Relapses occurred in 34, non-leukemic deaths in 77 (54%). Fifty-two patients died from documented (16 from fungi) and 18 from suspected (12 from fungi) infections. Causes of the documented fungal infections were: aspergillus in 12, candida in 2, cryptococcus and mucor. Six patients were on immunosuppressive therapy because of GvHD. LA decreased the probability of fungal-related mortality from 0.33 of the historical group to the present 0.20 (Wilcoxon p=0.01). Colonization at transplant and GvHD were the most significant risk factors (RR= 3.34 and 6.43, respectively). With regards to the side effects, there was a significant reduction (p<0.01) in frequency of infusion-related fever (11% vs 57%), chills/rigor (12% vs 65%), and cardiorespiratory events (5% vs 28%). Nephrotoxicity was almost completely prevented in all but 2 patients who developed severe renal failure. Three patients withdrew from AmBisome treatment because of back pain in two and anaphylaxis in the other. The LA-ambisome is an effective and well-tolerated anti-fungal prophylaxis for high-risk mismatched transplant recipients even at low doses.

CS012
PROGNOSTIC ROLE OF QUANTITATIVE MONITORING OF MIXED CHIMERISM IN ALLOTRANSPLANTED PATIENTS

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Transplantation of allogeneic bone marrow or peripheral hematopoietic stem cells is widely used to treat a variety of hematological disorders. In malignant diseases evaluation of chimerism after allotransplantation allows establishment of more efficacious and not-myeloablative conditioning regimens; prediction of the success or the failure of the graft; establishment of the timing of donor lymphocyte infusion in relapsed patients. In this paper we present results about 36 patients allo-transplanted for different malignancies tested by a fluorescent, fast, sensitive, specific and quantitative multiplex PCR method.
CS013
DONOR-RECIPIENT INCOMPATIBILITY AT CD31-CODON 563 IS A MAJOR RISK FACTOR FOR ACUTE GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENEIC BONE MARROW TRANSPANTATION FROM AN HLA-MATCHED DONOR

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The analysis of results of a large number of bone marrow transplants (BMT) from HLA-identical siblings clearly indicated that donor/recipient incompatibility for polymorphisms of molecules other than HLA antigens could be responsible for development of acute graft-versus-host disease (aGVHD). These antigens have been named minor histocompatibility antigens (mHA). A role for mHA has been proposed for the male-specific H-Y transplantation antigen, the HA-1 antigen, two polymorphisms of the CD31 molecule, and for some human platelet antigens (HPA). However, the relative importance of each polymorphism in eliciting aGVHD has never been tested at a clinical level in a single case series. To gain further information on this issue, we evaluated the correlation between aGVHD and donor/recipient incompatibility for HA-1, H-Y, CD31-codon 125, CD31-codon 563, HPA-1, HPA-3 and HPA-5 in 150 patients receiving BMT from an HLA-matched donor. Patients and methods. All patients (100 children and 50 adults) had hematological malignancies. Ninety-two were transplanted from a sibling, 58 from an unrelated donor. Investigated polymorphisms were typed by genom-techniques. Donor/recipient pairs were considered to be incompatible at HPA, CD31 or HA-1 when the recipient had an allele foreign to the donor. Logistic models were fitted to assess the association of polymorphisms and aGVHD, alone and after controlling for potential confounders. The joint effect of polymorphisms was judged by means of a multivariate logistic model. Results. Sixty-six out of the 150 patients (44%) experienced grade II-IV aGVHD. Univariate analysis showed that incompatibility for HPA-1, HPA-3, HPA-5, HA-1 and H-Y did not predict occurrence of aGVHD. The prevalence of aGVHD was higher in patients receiving a BMT from a donor incompatible for CD31-codon 125, but the difference did not reach statistical significance. By contrast, CD31-codon 563 incompatibility was a strong, statistically significant, risk factor for aGVHD (odds ratio, OR 3.97; CI 1.44-10.91; p = 0.008). For comparison, the risk of aGVHD deriving from a transplant from an unrelated donor was much lower (OR 1.93; CI 0.97-3.84; p = 0.06). To identify possible confounding factors interfering with the results of univariate analysis, a logistic model was applied, and the results of the univariate analysis were confirmed. Then, a multivariate model including BMT source and incompatibility for HPA-5, CD31-codon 125 and CD31-codon 563 was fitted. The results confirmed that aGVHD was more frequent when donor/recipient pairs were incompatible for CD31-codon 563 (OR 4.33; CI 1.38-13.51; p = 0.012) or when patients received bone marrow from unrelated donors (OR 1.93; CI 0.97-3.84; p = 0.037). Conclusions. Donor/recipient incompatibility for CD31-codon 563 is a strong risk factor for aGVHD and should be taken into account both for donor selection and adjustment of aGVHD prophylaxis in patients receiving allogeneic BMT.

CS014
AUTOLOGOUS STEM CELL TRANSPLANTATION IN UNRESPONSIVE MULTIPLE SCLEROSIS

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We designed a phase II clinical trial mainly directed to investigate MRI and laboratory changes following autologous PBPC transplantation in patients affected by advanced, poor prognosis and refractory MS. Only secondary progressive forms of MS were included. The study was approved by both the Italian Coop-
In a previous single-centre pilot study we observed a high rate of clinical and molecular remissions using the intensified high-dose sequential (i-HDS) chemotherapy regimen as first-line treatment for advanced follicle center lymphoma (FCL). To verify whether these results could be reproduced at a multicenter level, in 1997 we launched a study program among 20 Italian BMT Units. We used the original i-HDS protocol consisting of: i) debulking with two APO and two DHAP courses; ii) sequential high-dose (hd) delivery of etoposide, methotrexate and cyclophosphamide with peripheral blood progenitor cell (PBPC) harvest; iii) hd mitoxantrone + hd L-PAM followed by PBPC autografting; iv) consolidation radiotherapy on bulky sites. So far, 97 previously untreated patients have been enrolled by 20 Centers associated to the Italian Bone Marrow Transplant Group (GITMO). Main patient characteristics included: median age 45 years (range 28-59), stage IV=83%, leukemic disease (>12,000 lymphocytes/L), high LDH=34%, a.a. IPI score =2.5%=93%. At present, 87 patients completed the scheme and are evaluable. Two treatment-related fatalities (2.3%) were observed: one due to ventricular fibrillation and one due to CMV pneumonia. Five patients were not autografted due to patient refusal or low mobilization (5.7%). Second tumors are so far remarkably uncommon with only one patient (1.1%) developing acute T-lymphoblastic leukemia. There were 2 progressions under treatment (2.3%). Remission was partial in 8 patients (9.1%) and complete in 75 patients (86.2%). Molecular analysis could be performed at diagnosis on 73 patients. By the combined use of the t(14;18) and the IgH rearrangement we obtained a tumor marker in 79% of them. So far, 86 PBPC harvests have been analyzed and 48 (56%) resulted PCR negative. PCR-negativity at post-transplant follow-up was observed in 22 out of 36 patients studied (61%). Among PCR-negative patients only two relapses were observed, while ten relapses occurred among patients with persistent PCR-positivity (p=0.02). In conclusion: i) a hd-approach with PBPC autograft in FCL is feasible at a multicenter level; ii) results in terms of toxicity as well as clinical and molecular response are similar to those reported in single center studies. Based on these encouraging results, a randomized study comparing CHOP vs. i-HDS, both supplemented with Rituximab, has been recently started.
reported in NEJM (Aversa et al. NEJM 1998; 339:1186) received PBPCs depleted of T lymphocytes by one-step E-rosetting followed by a positive selection of the CD34+ cells with the CellPro device. Since January 1999 we have been using the CliniMacs instrument to select CD34+ cells in a one step procedure for 33 patients to date. All these extensively T-cell-depleted grafts contained large numbers of CD34+ cells (>10^6/kg). Since we introduced immunoselection of the CD34+ cells, we reduced the CD3+ cell contamination in the inoculum by one log (from a median of 2 × 10^5/kg to 3 × 10^4/kg). To reduce the extra-hematological toxicity of our conditioning regimen, in October 1995 we substituted fludarabine for CY and in January 1999 we decided to stop post-transplant G-CSF administration to recipients because of its immunosuppressive effect (Volpi et al. Blood 2001; 97:2514). Primary, sustained full-donor engraftment was achieved in 29/36 (80%) of the first study group and in 71 (94%) of the last 76 patients we have transplanted since October 1995. Furthermore, since we introduced the CD34+ cell selection, acute (3/73) and chronic (3/63) GvHD have been largely prevented. Overall, EFS at 7 years is 35% for AML and 13% for ALL patients. The patients who were in either bad-risk first CR (n= 7) or stable second CR (n=20) at the transplant have 0.50 probability of EFS at 7 years. Furthermore, TRM and EFS are even better if we analyze the 11 patients in stable CR II who benefitted from the changes we introduced in the last study (0.10 and 0.70, respectively).
chronic myeloid leukemia

CO001: CLINICAL AND MOLECULAR RESPONSE ANALYZED BY REAL-TIME QUANTIFICATION OF BCR-ABL TRANSCRIPTS IN CHRONIC MYELOID LEUKEMIA PATIENTS AFTER STI-571 THERAPY


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The knowledge that the transforming ability of BCR/ABL in several oncogenic pathways is principally due to its constitutive tyrosine-kinase (TK) activity has prompted new molecular therapeutic approaches. For example, the promising new anti-tumour TK activity has prompted new molecular approaches to the responsive patients. Taqman real-time RT-PCR proved a reliable and sensitive method for monitoring CML patients after STI-571 therapy. Indeed, we are able to report that the down-regulation in the bcr-abl/β2 microglobulin ratio recorded after hematological response to STI-571 was significantly stronger in those patients who achieved karyotypic response.

This study was supported by the Associazione Italiana per la Ricerca sul Cancro (A.I.R.C.), by MURST 40% (Sante Tura) (AML), by A.I.L., by the Italian 952206 C.N.R. target projects, by "30 Ore per la Vita" A.I.L. grants, and by CML Cofin 99 (M. Fiacchini, and G Saglio) fund.

CO002: STI571 IN THE TREATMENT OF PH+ CHRONIC MYELOID LEUKEMIA, IN CHRONIC PHASE: THE PRELIMINARY EXPERIENCE OF THE ITALIAN COOPERATIVE STUDY GROUP ON CML, IN PATIENTS RESISTANT TO OR INTOLERANT INTERFERON-α (PROTOCOL CML/002/STI571) ITALIAN COOPERATIVE STUDY GROUP ON CML


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The Italian Cooperative Study Group on CML between August, 2000 and February, 2001 has completed the recruitment time of a phase II, multicenter, observational study, aimed to evaluate the efficacy and safety of a inhibitor of the protein-tyrosine kinase Bcr-Abl associated, STI571, in adult patients with Ph+ chronic myeloid leukemia in chronic phase failing interferon-α for resistance or intolerance. The primary end-point of this study, of 12 months of duration, was the rate of major (1-35% Ph-) and complete (0% Ph-) cytogenetic response in patients who failed a interferon-α containing regimen according to 5 categories: 1) hematologic resistance: failure to achieve a stable hematologic response (up-front resistance) 2) cytogenetic resistance: failure to obtain an at least major (1-35% Ph-) cytogenetic response after 12 months of a interferon-α containing regimen, 3) cytogentic refractoriness: an increase in bone marrow Ph+positivity of at least 30% or above 65%, 4) hematologic refractoriness: a progressive leukocytosis on interferon-α and 5) patients intolerant to interferon-α. Moreover, among secondary objectives, the study scheduled the prospective evaluation of the molecular response to STI571, through the quantitative analysis of residual hybrid transcript on serial bone marrow and peripheral blood samples taken at fixed time points (6 evaluations in 12 months). Patients received STI571, 400 mg once a day, orally, except when adverse events required a dose reduction/interruption, for at least 12 months. A dose escalation to 600 mg/daily and 800 mg/daily was scheduled for patients resistant or refractory to STI571. Forty-six Italian hematology institutions enrolled 301 patients between August, 17th 2000 and May, 15th 2001. The preliminary results of this study will be presented.
C0003
NUCLEAR ENTRAPMENT OF THE BCR-ABL ONCOPROTEIN INDUCES
APOPTOSIS OF CHRONIC MYELOGENOUS LEUKEMIA CELLS

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Over 95% of patients affected by chronic myelogenous leukemia (CML) express the Bcr-Abl chimeric oncoprotein. Bcr-Abl presents three nuclear localization signals (NLS) and one nuclear export signal (NES) that should allow its continuous translocation to and from the nucleus. However, Bcr-Abl is found exclusively in the cytoplasm of leukemic cells. We have established that the cytoplasmic localization of Bcr-Abl is due to a lack of nuclear import. We have also discovered that inactivation of the Bcr-Abl kinase by point mutation can partially restore the import of Bcr-Abl to the nuclear compartment. The compound STI571 interacts with the ATP-binding lobe of both Abl and Bcr-Abl to inhibit their tyrosine kinase activity. Since a kinase defective mutant of Bcr-Abl can import to the nucleus, we hypothesized that STI571-mediated pharmacological suppression of the Bcr-Abl kinase might re-establish the nuclear import of the oncoprotein. Indeed, when we coupled STI571 with the drug leptomycin B (an inhibitor of nuclear export) we visualized part of the Bcr-Abl protein inside the nucleus of leukemic cells. Moreover, the reactivation of the nuclear Bcr-Abl kinase (by thorough washing or metabolic decay of the STI571) induced cell death. Thus, the combined treatment with STI571 and leptomycin B (LMB) causes the irreversible killing of Bcr-Abl-transformed cells, while each drug alone cannot achieve this effect. Furthermore, the combination of STI571 and LMB preferentially kills murine bone marrow cells expressing Bcr-Abl. The nuclear entrapment of an active Bcr-Abl kinase represents a potential new therapeutic approach tailored at the selective elimination of Bcr-Abl-positive leukemic cells.

C0004
ASSOCIATION OF α-INTERFERON AND AN ORAL FORM OF CYTARABINE
(CTARABINE OCTOFOSFATE - YNK01) IN THE TREATMENT OF PH-POS
CHRONIC MYELOGENOUS LEUKEMIA IN EARLY CHRONIC PHASE: RESULTS OF AN
ITALIAN COOPERATIVE STUDY GROUP ON CML (STUDY CML/97/YNK01)

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A phase II prospective multicenter protocol aimed to evaluate the efficacy of αIFN combined with YNK01 in patients with chronic myelogenous leukemia in chronic phase, Ph pos and/or bcr-abl pos, at diagnosis has been performed by the Italian Cooperative Study Group on CML. The endpoints were the hematological response rate at 3, 6 and 12 months, the cytogenetic response at 6-12 months and the evaluation of toxicity and compliance. The scheduled regime consisted of the administration of αIFN in escalating dose until 5 MUI/sm, if tolerated, and YNK01 600mg/d./14 days a month. The accrual started in January 1998 and ended in January 1999: a total of 90 patients were enrolled from 27 Italian Institutions. Risk profile according to Sokal or to the Euro score showed that more than half the population belonged to intermediate-high groups. Complete hematologic response was 67%, 74.5% at 3 and 6-12 months, respectively. Best cytogenetic response (complete + major, up to 33% Ph pos metaphases) was 14.5% and 26.5%. Hematological and cytogenetic response resulted very similar to those that have been reported for the LDAC+IFN arm of the ICSG randomized study started in 1994. Twelve patients received allogeneic BMT and one autologous BMT. Ten patient progressed to accelerated/blast phase. Ten patients went off protocol for toxicity. The most common side effects were gastrointestinal ones and were mainly due to YNK01. In the first month of therapy 29 diarrhea events were recorded and 21 of them were attributed to YNK01 by the investigators. Nausea and vomiting side effects were recorded in 45 cases in the first month while anorexia and weight loss were reported in 29 cases. Grade III-IV toxicity was reported in 10 patients. Three patients experienced grade IV toxicity (fever, CNS infection and hepatotoxicity); all these 3 pts are alive at last contact. Further detailed side effects and compliance data will be presented.

C0005
METHYLATION STATUS OF P15, P16 AND E-CADHERIN PROMOTERS IN
PRIMARY CHRONIC MYELOGENOUS LEUKEMIA CELLS

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Methylation of DNA is a biochemical modification that can influence gene expression and is involved in inactivating one of the two X chromosomes in women. Evidence that has recently accumulated suggests that cancer cells usurp this physiologic mechanism and use it to their benefit by inactivating tumor suppressor genes and related proteins. However, the primary structure of the affected proteins remains intact; reversal of abnormalities in DNA methylation may therefore restore the tumor-suppressive function of these genes and provide a novel approach to cancer therapy. The BCR-ABL chromosomal translocation is a central event in the pathogenesis of chronic myelogenous leukemia (CML). One of the ABL1 promoters (Pa) and the coding region of the gene are usually translocated intact to the BCR locus, but the translocated promoter appears to be silent in most cases. Recently, hypermethylation of Pa was demonstrated in CML and was proposed to mark advanced stages of the disease. Some years ago the hypermethylation of calcitonin gene was also demonstrated in CML. To study the role of hypermethylation in CML, the methylation status of 3 promoter-associated CpG islands was analyzed by PCR in 20 cases of CML treated in our institution. Cells were obtained from bone marrow and peripheral blood of CML patients at diagnosis and after 3, 6 and 12 months of treatment with either IFN or STI-571. After bisulfite treatment, modified patient DNA was amplified by PCR with specific primers both for methylated and unmethylated promoter sequences. Hypermethylation of e-cadherin, p15, p16 was relatively infrequent. For each of these CpG islands, the methylation density was not strictly correlated with stage of disease or...
response to therapy. Among patients who achieved a major cytogenetic response, low levels of methylation were associated with a trend towards improved survival, but this trend did not reach statistical significance. From our data, α-cadherin, p151, p16 methylation in CML does not appear to be associated with disease progression and it is not a predictor for survival or response to interferon-based therapy.

**CO006**

**AUTOGRRAFTING WITH MOBILIZED HEMATOPOIETIC PROGENITOR CELLS IN 99 PATIENTS WITH CHRONIC MYELOID LEUKEMIA**

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In these last eleven years we have autografted 99 patients in chronic phase CML. These patients were in late chronic phase and refractory to interferon-α (IFN-α) (n=49) (Group A) or in early chronic phase not previously treated with IFN-α (n=50) (Group B). All these patients were treated with ICE/mini-ICE protocols and G-CSF to mobilize hematopoietic stem cells. High dose therapy consisted of TBI-conditioning regimen (n=18) or protocols and G-CSF to mobilize hematopoietic stem cells. High dose therapy consisted of TBI-conditioning regimen (n=18) or TBI-conditioning regimen (n=18) or high-dose Busulfan (4 mg/kg/d for 4 days) (n=81). All patients were autografted with Ph-negative or <35% Ph+ mobilized hematopoietic progenitor cells. After engraftment, all patients received immunological therapy with IFN-α or IFN-α/IL-2, in the attempt to delay the blastic evolution. Sixty-eight (74%) patients are alive 2 to 105 months (median, 35 months) after ASCT. Twenty-three patients (group A: 18; group B: 5) have received immunological therapy with IFN-α or IFN-α/IL-2, in the attempt to delay the blastic evolution. Sixty-eight (74%) patients are alive 2 to 105 months (median, 35 months) after ASCT. Twenty-three patients (group A: 18; group B: 5) have developed blastic crisis from which all patients have died. Four patients of group B had an unrelated donor transplant and died of complications. One patient in group B with HBV+ died of fulminant hepatitis 4 months after autografting; two other patients in group A died of infection during aplasia. Thirty-five (35%) patients (group B: 31; group A: 4) are in complete/major cytogenetic remission at a median of 37 months (range, 9-99). All patients had some degree of stomatitis that was more severe in patients of group A. Gastrointestinal and hepatic toxicities were observed mainly in patients of group A. Thus, autografting with Ph-negative or <35% Ph+ mobilized hematopoietic progenitor cells can result in prolonged restoration of Ph-negative hematopoiesis for some patients with CML; moreover, most autograft recipients of Group B report normal or near normal activity levels, suggesting that this procedure when employed in the early phase of disease need not to be associated either with prolonged convalescence or with chronic debility.

**CO007**

**MDR1/P-gp EXPRESSION PREDICTS ACHIEVEMENT OF REMISSION IN ADULT ACUTE LYMHOBLASTIC LEUKEMIA PATIENTS**


The GIMEMA Cooperative Group, Rome

The prognostic relevance of multidrug resistance (MDR) overexpression has been reported in leukemia, especially in elderly patients affected by acute myeloid leukemia (AML) and in pediatric cases with acute lymphoblastic leukemia (ALL). Little information, however, is available on the role of the MDR gene (mdr1) and its protein (P-gp) in adults with ALL. The aim of our study was to evaluate in 363 newly diagnosed adult ALL patients, uniformly treated according to the GIMEMA 0496 protocol, the incidence and prognostic value of the MDR. The study was performed through a centralized handling of cell samples at presentation. Flow cytometric MDR1/P-gp expression (D-value) and rhodamine 123-efflux (Rhd123-E) was obtained in 203 and 158 prospective cases (minimum follow-up > 6 months), respectively.

P-gp expression ranged between 0 and 0.77, with a D-value ≥ 0.01 (MDR+) in 27.1% of cases. Functional efflux was found in 31.6% of cases who showed values ≥ 1.00 (range 0.38 - 3.66). Simple linear regression analysis showed a significant (p = 0.0001, r = 0.34) correlation between the two flow cytometric tests. In the 200 patients evaluable for response, MDR expression was not associated with clinical or pathologic characteristics. However, MDR1/P-gp expression significantly (p=0.01) predicts achievement of complete remission (CR) with a D-value that increases as the CR rate decreases. An even more significant association (p=0.0001) was found between achievement of CR and absence of MDR1/P-gp expression (80.8% of MDR patients achieved CR compared to 55.6% MDR+ patients). These results were confirmed in both B- and T-ALL subgroups. Functional studies, in addition, demonstrated the unfavorable role on achievement of CR (p=0.04) of higher Rhd123-E values. Multivariate analysis performed on 174 ALL patients showed that MDR1/P-gp expression (p=0.002), age (p=0.02) and CD34 expression (p=0.02) represented important independent predictors for response to therapy. Such data were confirmed by analysis of MDR1/P-gp expression as continuous variables on the overall population (p=0.05) and, more interestingly, in BCR/ABL negative ALL (p=0.02). Relapses occurred regardless of MDR1 expression. Probability of event-free survival (EFS) at 2 years differed significantly (p=0.003) between P-gp positive and negative patients: 21% vs. 31%, respectively. These results show that adult ALL expressing MDR1/P-gp are characterized by a lower likelihood of achieving CR and a shorter EFS duration, suggesting that such patients should be treated with regimens that do not include drugs involved in the MDR mechanisms and/or that include MDR-reversal agents.
A MULTIPLEX RT-PCR STRATEGY FOR RAPID MOLECULAR DIAGNOSIS OF THE MOST COMMON GENETIC LESIONS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA


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More than 50% of patients with acute lymphoblastic leukemia (ALL) presently show detectable genetic alterations that may provide independent prognostic factors of clinical outcome and guide the choice of different therapeutic options. Recently, Palisgaard et al. (Blood 1998; 92:574) proposed a multiplex RT-PCR analysis for a quick detection of the most common fusion genes occurring in patients with acute leukemias. In this study, in 114 ALL cases we used a modified version of this multiplex RT-PCR method, adapted to identify the alterations frequently occurring in ALL patients, and compared the observed results to those achieved by cytogenetic and Southern blot analyses. Forty-six patients were children and 68 adults (< 60 years). As a first step of our RT-PCR multiplex assay, two reaction tubes were set up to amplify the BCR/ABL p190 (e2a2) and p210 (b2a2, b3a2) isoforms, ALL1/AF4, ALL1/ENL (vial #1), and E2A/PBX1, TEL/AML1, SIL/TAL, HLFL/E2A (vial #2). Oligonucleotides to amplify the E2A were also added to each tube as an internal control. In addition, to avoid false positive results, a negative control, consisting of all reagents without RNA, was performed in each experiment. If an amplification product was observed in one tube, in the second step of the analysis a series of RT-PCR reactions were performed in order to confirm and identify precisely the specific fusion gene. Amplified products were observed in 47/114 cases (41%). With respect to patients’ age, a RT-PCR product was amplified in 19/46 (41%) pediatric patients and in 28/68 (41%) adults. In particular, among the 46 children, 2 patients (4%) expressed the BCR/ABL fusion gene, 7 (15%) the TEL/AML1, 3 (6%) the ALL1/AF4, 5 (10%) the E2A/PBX1 and 2 (4%) patients the SIL/TAL chimeric gene. In adults, a BCR/ABL positivity was observed in 20/68 (29%) patients, whereas ALL1/AF4 fusion was detected in 6 cases (9%), and TEL/AML1 and E2A/PBX1 chimeric genes were retrotranscribed in one case each. With respect to cytogenetic data, (t(9;22), (t(4;11), (t(12;21), (t(12;19) balanced translocations, which were always confirmed by multiplex RT-PCR. In addition, the first step of our multiplex RT-PCR system, which consisted of 4 reactions only, allowed rapid exclusion of the presence of the eight genetic alterations in 67/114 patients (59%). This procedure appears more cost-effective when compared to the conventional single lesions sequential PCR strategy, as it avoids performing more than 40 RT-PCR reactions necessary to evaluate these alterations individually. In conclusion, our data demonstrate that this multiplex RT-PCR strategy is a powerful method for a rapid and less expensive screening of the most common genetic alterations associated with ALL.

CLINICAL SIGNIFICANCE OF P-GLYCOPROTEIN EXPRESSION AND APOPTOSIS-RELATED PROTEINS IN ACUTE LYMPHOBLASTIC LEUKEMIA


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Multifactorial resistance mediated by P-glycoprotein (P-gp) and apoptosis-related proteins contributes both to chemotherapy failure and a worse outcome in acute lymphoblastic leukemia (ALL); however the exact prognostic impact of these resistance mechanisms is still unclear. In order to evaluate the clinical relevance of P-gp and bcl-2, we studied 87 de novo ALL patients, 46 males and 41 females, median age 36 years (range 1-73) from 1988 to 2000. Forty out of 73 adult patients were treated with a conventional regimen (protocol 0288 GIMEMA), whereas 33 patients underwent intensive chemotherapy based on high-dose cytarabine and mitoxantrone (HDAraC/MTZ), followed by a transplantation procedure. Fourteen pediatric patients were treated according to the AIEOP protocols. After incubation with C219 or JSB-1 or anti-bcl-2 monoclonal antibodies (MoAbs), the expression of P-gp (C219 and JSB1) and bcl-2 was measured by flow cytometry. The results were obtained as mean fluorescence index (MFI), expressed as the ratio of sample mean channel: control mean channel. The thresholds were set at > 2 for C219, at ≥ 5 for JSB-1, at ≥ 15 for bcl-2; 39.1% of the patients were C219 positive, 29.7% JSB-1 positive and 54.6% bcl-2 positive. No significant correlation was observed between P-gp levels (C219 and JSB-1) and WBC count, B or T immunophenotype, CD34 or myeloid antigen expression. On the other hand, significant associations were observed between higher bcl-2 protein levels and age younger than 45 years (p=0.011), WBC count lower than 50 × 10^9/L (p=0.039), CD34 expression (p=0.018), and normal karyotype (p=0.018). With regard to complete remission rate (CR), no significant difference was found between P-gp positive and P-gp negative patients. Moreover, a longer overall survival (OS) and disease-free survival (DFS) was observed in P-gp negative patients (p=0.013 and p=0.015 for C219 and p=0.008 and p=0.0002 for JSB-1). Furthermore, bcl-2 positive patients showed a higher CR rate (91.5% vs. 69.2%; p=0.008), a longer OS (p=0.002) and DFS (p=0.013). No significant correlation was found between P-gp and bcl-2 levels and relapse rate. Besides, JSB maintained its prognostic significance with regard to OS (p=0.03) and DFS (p=0.016 and p=0.005) within the patient groups treated with 0288 and HDAraC/MTZ protocols, whereas only bcl-2 showed a strong prognostic impact on the OS (p=0.002) and DFS (p=0.004) within the pediatric subset. In multivariate analysis JSB-1 was found to be an independent prognostic factors with regard to OS (p=0.04) and DFS (p=0.0009) together with cytogenetics and age. The favorable prognostic impact of higher bcl-2 levels demonstrates that high apoptotic levels are significantly associated with a worse outcome in ALL. In conclusion, P-gp determination may be added to the other well-known biological factors in order to better stratify the risk groups in ALL.
PREVALENCE OF FLT3 INTERNAL TANDEM DUPLICATION IN ADULT ACUTE MYELOID LEUKEMIA

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Flt3 belongs to the class III tyrosine kinase receptor family that includes c-kit, c-fms, and PDGF receptors. Flt3 is expressed on hematopoietic progenitor cells and mediates proliferation and differentiation. An internal tandem duplication of the Flt3 gene (Flt3/ITD) has been reported in acute myeloid leukemia (AML) which may be associated with poor prognosis. The aim of this study was to evaluate the prevalence and the prognostic significance of this common molecular alteration in adult AML consecutively registered at our institution from 1994 to 2000. To avoid possible selection bias, RNA or DNA were obtained not only from freshly isolated or frozen cells but also from diagnostic or diagnostic slide smears. By PCR analysis we could analyze pathologic samples from 209 AML patients with a median age of 55 (range 3–90). Fifty-two patients were positive for Flt3/ITD (22%). In all the sequenced amplified duplications we could confirm that all of them involved exon 11 and preserved the open reading frame. By univariate analysis we could demonstrate that no significant difference in the frequency of Flt3/ITD can be established according to FAB, sex and age. However, we confirm that Flt3/ITD is significantly more frequent in patients with a high leukocyte count at diagnosis since in positive patients the median leukocyte count was 38 × 10^9 as compared to 7.7 × 10^9/L. For patients treated with curative protocols, the complete remission (CR) rate was 67% among negative patients and 55% in Flt3/ITD positive patients and a significantly better overall survival was shown in negative patients (p = 0.05). By multivariate analysis, a high leukocyte count and Flt3/ITD were confirmed to be independently associated with a remarkably poor prognosis in AML patients. Ongoing analyses are currently aimed to evaluate the prognostic value of Flt3/ITD when considering other biologic and cytogenetic risk factors and the effect of different therapeutic protocols.

BUTYRATES RECRUIT TRANSCRIPTIONAL REPRESSION AND INDUCE MATURATION OF ACUTE MYELOID LEUKEMIA LEUKEMIA CELLS

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Dysregulation of histone acetylation has been demonstrated in several hematological neoplasias. Myeloid maturation is directed by a complex transcriptional program. In t(8;21) AML, a stable association of AML1/ETO fusion protein with the nuclear histone deacetylase complex (HDAC1) is crucial to repress transcription of AML1 target genes and to block differentiation of hematopoietic precursors. We investigated whether butyrate-stable monosaccharide derivatives could reverse HDAC-mediated repression and we also evaluated whether butyrate could act synergically with G-CSF and/or ATRA in inducing myeloid differentiation. We already demonstrated that butyrate-stable monosaccharide derivatives (D1= O-n-butanol-2,3-O-isopropilidene-α-D-manno-furanoside and G1= 1-O-n-butanyl-DL-xyitol) were able to provoke maturation and apoptosis in AML blast cells. Butyrates are known to be specific and potent inhibitors of HDACs. We evaluated the biological response to butyrates of Kasumi-1 cell line, derived from an AML-M2 patient with t(8;21) translocation and expressing the AML/ETO fusion protein. In parallel we exposed to butyrate derivatives (0.5 and 1mM) for five days in standard culture conditions and serum-free medium, primary cultures of AML blasts obtained from 5 t (8;21)
AML cases. Butyrate treatment caused inhibition of proliferation, accumulation of AML blast cells in G1 phase of cell cycle and increased expression of specific myeloid differentiation markers. Flow cytometric analysis of annexin-V positive cells showed induction of programmed cell death. To investigate whether the effect of butyrate was correlated with modifications of histone acetylation in Kasumi-1 cells and primary AML blasts, we evaluated the status of acetylation of histone H4 by Western blotting analysis using specific antibodies directed towards the acetylated form of the histone. In untreated AML blasts acetylated histone H4 was quantitatively negligible, whereas five day exposure in liquid culture with monosaccharide butyrate as single agents induced its acetylation. This phenomenon was significantly enhanced by addition of G-CSF and/or ATRA to cultures containing butyrate derivatives. Caspase 3 and caspase 9 expression was also analyzed after butyrate derivative treatment. Caspase 9 was cleaved after 72 hrs butyrate monosaccharide exposure and caspase 3 was activated. These results suggest that myeloid differentiation induced by butyrate (and G-CSF and/or ATRA) might be a consequence of a double effect: transcriptional derepression by butyrate and possibly transcriptional activation by G-CSF and ATRA. Our data suggest the possibility of removing AML typical block of maturation by transcriptional/differentiation therapy, effective on modifying specific molecular alterations of myeloid maturation pathway such as those present in t(8;21) AML.

RESULTS

Continuous complete remission (CCR) and disease-free survival (DFS) were 2.2, 2.4 and 2 years, respectively. PDN-pretreatment response resulted the main independent factor influencing CR achievement; OS, CCR and DFS. Neither induction intensification nor early consolidation appeared to influence CCR and DFS duration. With respect to our previous ALL 0183 trial, in this study a slight improvement of long-term DFS (at 9 yr 29% vs 25%) was achieved. We could not demonstrate an independent role for immunophenotype: B lineage show a marginally better outcome; as compared to B lineage, T lineage ALL were less responsive to PDN-pretreatment but CR rate was slightly higher (83% vs 85%). However, this good induction result did not translate into a higher percentage of long-term CCRs and DFSs. For the first time, in adult ALL patients PDN-pretreatment response proved to be a powerful factor predicting disease outcome.

CO013

GIMEMA ALL0288 RANDOMIZED STUDY: RESULTS OF LONG-TERM FOLLOW UP

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The GIMEMA ALL 0288 trial was activated to evaluate: (1) the impact of a 7-day prednisone (PDN) pretreatment on CR achievement and length; (2) the influence of addition of cyclophosphamide (Cy+) (Random I) to a conventional 4-drug induction on CR rate and duration; (3) whether an early post-induction intensification (Random II) by an 8-drug consolidation could improve CR duration. From May 1990, according to a pilot study in the context of this trial, PH+ patients underwent bone marrow transplant (Mandelli, 1992). Median follow-up of this study is 7.3 years. From January 1988 to April 1994, of 794 patients were responders and 35% non-responders. CR was achieved in 627 patients (82%), resistant patients and induction death rate was 11% and 7%, respectively. No difference between the two randomized induction arms was found concerning CR rate (81% in Cy+ and 83% in Cy-), resistant patients and induction death. As for PH+ ALL patients, 26 (72%) resulted PDN-responders and 39/47 (83%) achieved CHR. Random II was applied to 388 CRs: 201 had maintenance alone, 187 consolidation followed by maintenance. Relapse rate was 60%; isolated CNS relapses were 8% of all relapses and 13% of all relapses. Median survival (OS), continuous complete remission (CCR) and disease-free survival (DFS) were 2.2, 2.4 and 2 years, respectively. PDN-pretreatment response resulted the main independent factor influencing CR achievement; OS, CCR and DFS. Neither induction intensification nor early consolidation appeared to influence CCR and DFS duration. With respect to our previous ALL 0183 trial, in this study a slight improvement of long-term DFS (at 9 yr 29% vs 25%) was achieved. We could not demonstrate an independent role for immunophenotype: B lineage show a marginally better outcome; as compared to B lineage, T lineage ALL were less responsive to PDN-pretreatment but CR rate was slightly higher (83% vs 85%). However, this good induction result did not translate into a higher percentage of long-term CCRs and DFSs. For the first time, in adult ALL patients PDN-pretreatment response proved to be a powerful factor predicting disease outcome.

CO014

FLOW-CYTOMETRIC DETECTION OF MINIMAL RESIDUAL DISEASE IN ADULT T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Immunophenotypic detection of minimal residual disease (MRD) in childhood acute lymphoblastic leukemias (ALL) can identify patients with high risk of relapse and poor outcome. The small number of suitable cases in each center limits the assessment of the effectiveness of this method in adult ALL. Therefore, co-operative studies with centralized sample analysis may be useful to supply further information. We analyzed MRD by flow-cytometry using a leukemia-specific marker combination (cytoplasmic CD3/nuclear TdT) in 45 of 93 T-ALL patients, all treated according to the Italian multicenter GIMEMA LAL 0496 protocol between April 1996 and January 2001. The analysis of bone marrow samples for MRD detection was carried out in only one center for all patients. Results. 23/45 (51.1%) relapsed, 6/45 (13.4%) were refractory to treatment and 16/45 (35.5%) are at present in continuous complete remission (CCR). The most relapse-predicting MRD controls were at 2 months (pre-consolidation) and at 8-10 months from diagnosis (pre-6th reinduction cycle), as indicated in the Table below. Conclusions: Flow-cytometric detection of the leukemia-specific cytoplasmic CD3/nuclear TdT marker combination had a predictive value in most cases of relapsed adult T-ALL. Our data further support the evidence that immunophenotypic MRD monitoring is a useful outcome predictor also in adult T-ALL.

<table>
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<tr>
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<th>1st EVALUATION (1st consolidation)</th>
<th>Median % of ccCD3/nuTdT+cells</th>
<th>Probability of relapse at 3 year</th>
<th>95% CI</th>
<th>p-value</th>
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<tbody>
<tr>
<td>MDR- Patients (n=37)</td>
<td>0.005% (0.005-0.027)</td>
<td>15.5%</td>
<td>10.9-20.1%</td>
<td>&lt;0.01</td>
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<tr>
<td>MDR+ Patients (n=7)</td>
<td>0.45% (0.18-1.3)</td>
<td>19.5%</td>
<td>10.9-29.6%</td>
<td>&lt;0.05</td>
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</table>

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<thead>
<tr>
<th>2nd EVALUATION</th>
<th>Median % of ccCD3/nuTdT+cells</th>
<th>Probability of relapse at 3 year</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st EVALUATION (1st consolidation)</td>
<td>0.47% (0.01-1.0)</td>
<td>15.5%</td>
<td>7.9-23.1%</td>
<td>&lt;0.032</td>
</tr>
<tr>
<td>2nd EVALUATION</td>
<td>0.005% (0.005-0.027)</td>
<td>13.5%</td>
<td>11.5-15.5%</td>
<td>&lt;0.001</td>
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Adult ALL can be cured by chemotherapy in less than 30% of cases, because of the high relapse rate. The role of bone marrow transplantation for ALL patients in first CR has not been definitively addressed. In 1998, based on the MSKCC experience, a multicenter phase II protocol for resistant and relapsed adult ALL patients was activated with two main objectives: i) to evaluate CR rate after high dose chemotherapy; ii) to evaluate bone marrow transplant feasibility and efficacy. Treatment schedule consisted of: ARA-C 3 g/m² d 1-5; idarubicin 40mg/m² on day 3; G-CSF from d+7. Patients in CR were treated with one or more courses of vindesine, HD-MTX and dexamethasone to consolidate response, and then addressed to a transplant procedure. Seventy-seven patients (43 males, 34 females) were enrolled from 13 GIMEMA Institutions; median age was 42 y (range 16-69). Sixty-three patients were transplanted: 1 from autologous PBSC; 12 from an HLA-identical sibling; 5 from MUD; 4 from haploidentical donor; 38 from unrelated donors; 10 from cord blood. A preliminary analysis has shown an overall complete remission (CCR) of less than 10%. No difference was observed among patients enrolled for resistant or relapsed disease; transplanted patients showed a better survival (CCR 25% at 30 mo.) compared with patients in CR not eligible for a transplant procedure. We conclude that: i) high dose chemotherapy is effective in resistant or relapsed ALL patients; ii) a single high dose idarubicin is well tolerated, without significant toxicity; iii) bone marrow transplantation gives encouraging results in this subset of poor prognosis patients.
oral communications - acute leukemias

notype, blast count < 10,000, and negative t(9;22)/ t(4;11). The SR protocol comprised intensive early anthracyclines (total idarubicin 96 mg/m²) and no transplant. HR patients were eligible for different options: allograft from HLA-compatible sibling; autograft (no donor) after a short high-dose sequence with cyclophosphamide, ara-c, methotrexate, and melphalan plus TBI (the blood cell autograft being purged immunomagnetically); or T-lineage protocol with high-dose ara-c and increased cyclophosphamide and methotrexate. Cases of HR B-lineage ALL unfit for high-dose treatment were shifted to SR protocol. According to treatment intention, 93% of SR patients and 82% of HR patients were consolidated with SR and HR protocols, respectively; 2 cases received no post-remission therapy and 13 HR were shifted to the SR program. DFS results at 4 years were as follows:

<table>
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<tr>
<th>Group</th>
<th>DFS at 4 years</th>
</tr>
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<tbody>
<tr>
<td>All patients</td>
<td>48%</td>
</tr>
<tr>
<td>SR patients</td>
<td>64%</td>
</tr>
<tr>
<td>HR patients</td>
<td>42% (p=0.05)</td>
</tr>
</tbody>
</table>

DFS rates in HR patients treated with different options were: Allograft (n=21) 42%, Autograft (n=29) 32%; shift to SR (n=13) 54% and T-cell protocol (n=10) 58%. These results were not significantly different and the only prognostic variable associated with an unfavorable outcome was Ph/BCR- ABL positivity. This study documents the value of anthracyclines in SR ALL. The role of high dose transplant procedure in HR subsets remains to be elucidated.

**CO018**

**TREATMENT OF ELDERLY PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA. THE GIMEMA EXPERIENCE**


GIMEMA A Cooperative Group, Roma

Background. The introduction of all-trans retinoic acid (ATRA) as tailored treatment for acute promyelocytic leukemia (APL) has markedly improved the overall results in this subtype of acute myelogenous leukemia (AML). As advanced age is one of the most important adverse prognostic factor in AML, we revised elderly APL patients enrolled in the GIMEMA - AIDA protocols to evaluate the impact of ATRA in this poor prognostic subgroup. Material and methods. One hundred and thirty-six patients with newly diagnosed, de novo α-positive APL aged > 60 years were enrolled in two consecutive GIMEMA protocols; from 1/93 to 1/96 in the standard AIDA (s-AIDA) protocol (Mandelli et al., Blood 1997), from 1/97 to 12/2000 in age-adapted modified AIDA regimen (m-AIDA), including the same induction as s-AIDA to follow the impact of ATRA in this poor prognostic subgroup. Results: Of 130 evaluable patients (M/F 71/59, median age 65.7 years, range 60 - 74.8, median WBC 1.8 × 10⁹/L, range 0.3 - 122.4), 112 (86.5%) achieved CR, 16 (12%) died during induction and 2 (1.5%) were resistant. As to consolidation, only 48/99 patients in both protocols completed the 3 consolidation courses. Of these, 37 were excluded after the 1st course (22 according to the AIDA-m protocol, 12 for toxicity and 3 for relapse) while 14 were excluded after the 2nd consolidation course (13 for toxicity and 1 for relapse). Sixteen relapsed and 15 died in CR of treatment-related toxicity (4 closely after induction treatment and 11 during consolidation courses). The 5-year CCR and DFS in the whole population were 74% and 62%, respectively. There were no statistically significant differences in outcome results among patients who received 1, 2 or 3 consolidation courses. Conclusions: The inclusion of ATRA in front-line therapy markedly improves APL prognosis also in the elderly, with CR and DFS rates similar to those achieved in younger patients and considerably higher than those of elderly patients with other AML subtypes. Our results also suggest that a less intensive treatment plan (m-AIDA) is equally effective in elderly APL patients, allowing the severe treatment-related toxicity commonly reported in this category to be reduced.

**CO019**

**ACUTE MYELOID LEUKEMIA: FLAG REGIMEN AS INDUCTION THERAPY FOR PREVIOUSLY UNTREATED ELDERLY PATIENTS**

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The incidence of acute myeloid leukemia (AML) is prevalent in adults patients and its incidence increases with rising age. More than 50% of the patients (pts) with AML are over 60 years of age. We describe our experience of 51 elderly (>60 years) pts. suffering from de novo (33) or secondary untreated AM L (18) by fludarabine (30 mg/m² i.v. day 1 to 5) Cytarabine (2 g/m² i.v. day 1 to 5) and G-CSF which was administered daily at the dose of 5 mg/kg sc. from the day-1 until neutrophil recovery > 1×10⁹/L (FLAG protocol). Median age was 66 years (range 60-77), 31 pts were males and 20 females. WHO performance status (PS) was grade 1 in 31 pts, grade 2 in 18 and grade 3 in 2 pts. Twenty-nine out of 51 pts (56.8%) achieved CR after only one course of FLAG. Seven pts died during the hypo-aplastic phase of cerebral hemorrhage (4) or sepsis (3); 13 (25.5 %) had resistant disease. Two patients showed partial remission (PR). De novo and secondary AML patients showed 69% and 33% CR rates, respectively. All patients in CR received a second course of FLAG regimen as consolidation, the other pts were considered off study. Patients in PR were observed with supportive therapy and oral chemotherapy (OS 16 and 3 months). All patients experienced profound cytopenia. Median time to neutrophil >500(µL recovery was 18 days (range 14-26), while a platelet count >20000/L was reached after 19 days (range 14-29). The median period of hospitalization was 22 days (range 11-53). The non-hematological toxicity was mild. The median CR duration was 8 months (range 2-10). Currently 17 (33%) patients are alive, but 13 pts maintained continuous CR (CCR). Overall, in 14 pts relapse occurred 1-14 months after CR. One patient died in CR for sepsis after the consolidation cycle. Overall median survival (OS) of these 51 pts was 8.5 months (range 1-29). Following CR achievement, 4 pts received autoBMT (2 pts with bone marrow, 2 pts with CSSP); 2 pts relapsed and died, 2 pts were in first CCR at +1 and +13 months after transplantation; ten pts received monthly cycles with oral idarubicin plus low dose ARA-C or thioguanine or etoposide for six courses. The results of the current study demonstrate that the FLAG regimen offers high rates of CR in patients with de novo AML with acceptable toxicity; the low toxicity after induction and consolidation therapy allows transplantation opportunities in some elderly patients.
CO020
PROGNOSTIC IMPACT OF CD56 IN 141 CASES OF ACUTE MYELOID LEUKEMIA

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All the FAB cytotypes, and in particular M4 and the acute myeloid leukemia (AML) originating in a common precursor myeloid/NK, can express CD56. Whilst the negative prognostic related to CD56 in M2 with t(8;21) and in M3 is known, there is no definite evidence in AML in general. We hereby outline the results regarding 141 consecutive cases of AML at diagnosis under our observation from January 1997 to February 2001, with a follow-up on the 31st of March 2001. The aim of the study was to evaluate the expression of CD56, its prognostic relevance and the clinical characteristics of the CD56(+) cases. The patients' clinical characteristics were: 65M/76F, median age 59 yrs, 49 were the cases with WBC ≥ 40 x 10^9/L FAB cytotype: 9 M0, 25 M1, 35 M2, 11 M3, 47 M4, 11 M5, 2 M6, 1 M7. 90 were de novo AML, 31 post-MDS and 20 s-AML. In the 118 cases with evaluable karyotype, 21 were at a favorable prognosis, 65 intermediate and 31 unfavorable. CD56 was positive (≥ 20% of the blasts) in 28 patients (20%) without difference in age, cytotype, CD34(+) or WBC at diagnosis regarding the CD56(+) cases. Instead, the occurrence of CD56(+) was significantly higher in the s-AML (p=0.0001). The karyotypes at unfavorable and intermediate prognosis were equally distributed in the CD56(+) and CD56(−) patients, whereas, more occurrences of favorable karyotypes were noticeable in the CD56(+) and CD56(−) patients, group. Eighty-seven patients underwent bone marrow transplant, 29 in the CD56(+) group, 8 in the CD56(−) one.

The expression of CD56 does not have a relevance on obtaining complete remission, which was similar in the two groups. Instead, a significant worsening of disease and event-free survival was observed in the CD56(+) patients. Whereas the event-free survival in the CD56(−) patients reached a plateau of 33% in 3 years, that of CD56(+) cases reached zero after 18 months. Regardless of a sufficient number of registered events (70%), these results need to be confirmed after a longer follow-up.

CO021
FLAG-IDA + STEM CELL TRANSPLANTATION, A FEASIBLE AND EFFECTIVE TREATMENT FOR DE NOVO ACUTE MYELOID LEUKEMIA PATIENTS (< 60 YEARS): PRELIMINARY RESULTS OF A PROSPECTIVE SINGLE CENTER STUDY

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Introduction. Autologous and allogeneic progenitor cell transplantation might improve the outcome of younger AML patients. However, high dose therapy in first CR can be delivered to only 1/3 of the potentially eligible patients due to disease progression under therapy, early relapse, or chemotherapy-related toxicity. The choice of a regimen which includes synergistic drugs such as intermediate dosage Ara-C, idarubicin and fludarabine might reduce treatment failure. The relatively short duration of chemotherapy might, on the other hand, reduce toxicity and allow prompt and safe administration of high dose therapy. Patients and methods. The regimen (FLAG-Ida) included fludarabine (30 mg/m^2), followed four hours later by a 2-hour infusion of Ara-C (2 g/m^2) on days 1-5, and by a 30 minute infusion of idarubicin (10 mg/m^2) on days 1,3,5. G-CSF (300 mcg/day) was administered s.c. 12 hours before starting fludarabine and was continued for five days. High dose therapy with stem cell rescue was planned for all patients in first CR after consolidation course and in good clinical conditions. Forty-three consecutive patients with de novo and untreated non-M3 AML entered the study between June 1995 and February 2001. The mean age of patients was 50 (range 15-59). Results. Non-hematological toxicity of FLAG-Ida was very low. The median time to PMN recovery (> 0.5 x 10^9/L) was 17 days (range 10-28) and 50 x 10^9/L platelets were reached at a median of 17 days (12-38). During the neutrophenic phase Gram+ or Gram- bacteria were isolated from the blood in 7 patients. Pneumonia occurred in 3 patients and pulmonary aspergillosis in 1. Forty-one (95%) patients were evaluated for response. One patient died during induction of Trichosporon asahii sepsis (2%). In another patient the response had not been evaluated yet. CR was achieved in 35 patients (81%) while 6 patients had resistant disease (14%). Only one patient could be salvaged and is still in CR at 65 months from the beginning of therapy. Nine-teen patients underwent autologous (N = 14) or allogeneic (N = 5) bone marrow or PBPC transplantation. Twenty-three patients are alive. Twenty have died (1 during induction, the others during disease progression). After a mean follow-up of 20 months, the average duration of CR is 14 months (range 2-48) and the mean survival is 17 months (range 1-65). The 4 year projected DFS and overall survival (OS) were 32% and 43%, respectively. Among patients undergoing stem cell transplantation DFS and OS were 53% and 59%. Good-intermediate and unfavorable karyotype patients were significantly different as far as OS (45% vs 0%; p 0.000002921) and DFS (38% vs 0%; p = 0.00134) are concerned. Conclusions. In conclusion, FLAG-Ida proved to be effective and well tolerated when employed as front line induction therapy in patients aged 60 years or less. As expected, outcome was better than what has been reported in secondary, refractory, and relapsed AML. Furthermore, the subsequent intensification with allogeneic and autologous transplantation was feasible and well tolerated.
Hodgkin’s disease

ELEVATED SERUM LEVELS OF THE SOLUBLE FORM OF CD30 MOLECULE ARE ASSOCIATED WITH INFERIOR EVENT-FREE SURVIVAL IN HODGKIN’S DISEASE PATIENTS

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Serum concentration of the soluble form of CD30 molecule (sCD30) is often increased in Hodgkin’s disease (HD) patients. The molecule is likely to play a pathogenetic role in this disease, both functioning as a transmembrane cytokine receptor, and interacting with its ligand which is involved in the growth-regulation of HD-derived cell lines. We previously reported that a high circulating level of sCD30 is independently associated with an inferior event-free survival (EFS) in patients with HD. In this study we evaluated the role of pre-treatment serum level of sCD30 in a large series of patients with HD from five different international institutions. We identified 595 previously untreated patients who presented to the participating centers between 1984 and 1999, and had available sera for the determination of sCD30 levels. Median age was 34 years (range 12-82), and gender was male in 56%. Ann Arbor stage was I in 12%, II in 52%, III in 19%, and IV in 17%, and B-symptoms were present in 38% of the patients. Histology was lymphocyte predominance in 7%, and classical HD encompassing nodular sclerosis in 73%, mixed cellularity in 16%, lymphocyte depleted in less than 1%, and unclassified in 3%. Treatment consisted of radiotherapy in 17%, chemotherapy only in 33%, and combined modality therapy in 50% of the patients. Observed median serum level of sCD30 (39 U/µL, range 0-3610) was significantly higher (p<0.001) when compared with a group of 113 healthy controls (4 U/µL, range 0-20). Levels were considered high (>100 U/mL) in 25% of patients. Higher levels of sCD30 significantly correlated with advanced stage, presence of B-symptoms, bone marrow involvement, large mediastinal mass, elevated serum levels of LDH, high β2-microglobulin, and with low levels of serum albumin. With a median follow-up of 76 months for survivors, the actuarial 10-year EFS for all patients was 75±2% (±SE). High sCD30 levels were significantly associated with poor outcome in our patients, and the 10-year EFS for patients with sCD30 levels ≥100 vs <100 U/µL was 59% vs 80%, respectively (p=0.0001). Our data confirm the possible role of sCD30 measurement in identifying HD patients at higher risk of relapse, who may take advantage from investigational therapies.

CO023
IGEV: A PROMISING PRE-TRANSPLANT REGIMEN IN RELAPSED/REFRACTORY HODGKIN’S DISEASE

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With the aim to induce both optimal cytoreduction and CD34+ cell mobilization in patients (pts) with pretreated Hodgkin’s disease (HD) candidates for high-dose chemotherapy (HDCT) consolidation with peripheral blood stem-cell (PBSC) rescue, we tested the IGEV regimen, consisting in ifosfamide 2000 mg/m² iv infusion day 1-4 with MESNA uroprotection, gemcitabine 800 mg short iv infusion day 1 & 4, vinorelbine 20 mg/m² iv push day 1, and prednisolone 100 mg iv day 1-4. Cycles were given every 21 days, with G-CSF 300 micrograms sc from day 7 to day 13 of each cycle, or up to the apheresis day(s). Four cycles of IGEV were planned, and leukapheresis was performed after the 3rd or 4th course, provided at least partial remission (PR) was achieved after the 2nd course. Responding pts proceeded to HDCT with thiotepa (600 mg/m² day -3) and melphalan (140 mg/m² day -1) with PBSC reinfusion on day 0. From 10/98 to 03/01 29 pts. (21 males/8 females) with a median age of 30 years (range 18-59) have been accrued. Nine had primary refractory and 20 relapsed disease. All had previously received at least one CT regimen, and nine extended-field radiotherapy as well. Among 27 pts assessable for response, 12 achieved complete response (CR) and 12 PR (possibly CR-u in four cases), for an overall response rate of 88%, while three did not respond. Myelosuppression of mild to moderate intensity was the most common adverse event: grade III-IV neutropenia occurred in 29% of 94 evaluable cycles, thrombocytopenia below 50,000/m3 in 28% with only one pt requiring platelet transfusion, whereas red cell support was needed by two pts. Four courses were delayed because of myelotoxicity. Non-hematological side-effects were minimal and neither treatment-related death nor hospitalization related to toxicity occurred. As detailed elsewhere, in all cases but one an adequate amount of CD34+ cells to support myeloablative chemotherapy could be collected. At present, 18 pts have received HDCT with PBSC support. HDCT converted PR in CR in five pts, and thus at the end of the treatment program 17 were in CR. Four pts have relapsed or progressed. After a median follow-up of 12 months (range 3-36), 11/18 autotransplanted pts are alive and disease-free, (one after relapse and salvage RT), one is alive with disease, five have died (three of lymphoma and two in CR from other causes, probably related to transplant procedure in one case). In conclusion, IGEV is a very effective cytoreductive and mobilizing regimen with acceptable toxicity in pts with refractory/relapsed HD eligible for PBSC-transplantation. Further escalation of IGEV doses in order to increase CR rate, as well as the tandem HDCT procedure are under evaluation.
VBM + IFRT IN EARLY STAGE HODGKIN’S DISEASE

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Conflicting results are available on VBM + IFRT in treatment of early stage HD patients. Despite high response rate and good clinical results in terms of EFS and PS, unacceptable hematological and pulmonary toxicity has been reported by some English and Italian groups. We reported our experience on treatment of early stage HD patients with three cycles of VBM and IFRT followed by a further three cycles of VBM with dose reduction of bleomycin. Forty-four pts with histologically proven HD, stage I-II, were treated in two different Institutes. Twenty-two females and 22 males with a median age of 31 years (17 - 76) with PS not exceeding 2 according to the ECOG scale, received 3 cycles of vinblastine 6 mg/m2, bleomycin 10 IU/m2, methotrexate 30 mg/m2 i.v. day 1-8 followed by IFRT and 3 additional cycles of VBM with dose reduction of bleomycin. All pts were evaluated before, during and after treatment with CT scan, clinically and with laboratory tests. All pts performed pulmonary function tests at the end of treatment. In brief, 42/43 pts (98%) achieved a clinical and radiological CR. Seven pts relapsed in the first three years from the end of therapy. Five of them were still alive and in CR after savage therapy. With a median follow-up of 3 years, 92% of pts are alive and free of disease. No pts experienced acute and late pulmonary toxicity and among 184 cycles evaluated, 29 (15%) episodes of severe neutropenia were recorded. Only 1 pt experienced febrile neutropaenia and no toxic death was reported. So that VBM + IFRT + VBM in our experience seems to be safe and feasible. Clinical results achieved suggest such treatment as suitable for early stage HD principally for those pts with good prognostic factors. Finally, the next step could be to evaluate four cycles of VBM followed by IFRT in order to reduce the length of treatment.

ANALYSIS OF THE PREDICTIVE ABILITY OF THE PROGNOSTIC MODELS DEvised FOR ADVANCED HODGKIN’S DISEASE. A PROPOSAL FOR THE INTEGRATION OF THE BEST THREE SYSTEMS


Defining different risk groups to differentiate the intensity of work-up and therapy is becoming increasingly important in Hodgkin’s disease patients. Several prognostic systems have been elaborated over the last 12 years, but early identification of a reasonably large group of low- or high-risk advanced-stage patients remains unsatisfactory. Seven distinct well-known models were applied to 516 patients with advanced HD, 315 of whom were taken as the study sample (145 enrolled by the Italian Lymphoma Study Group – GISL – between 1988 and 1994; 170 treated in the Institute of Hematology of the University of Bologna between 1979 and 1994) and 201 as the test sample (recorded by the AIL-Linfomi from 1980 to 1985). The models were the following: the equation of the University of Pavia and Modena, the index of the St. Bartholomew’s Hospital and Christie Hospital, the model of the Memorial Sloan Kettering (MSK), the equation of the Scottish and Newcastle Lymphoma Group, the model drawn by the International Database on Hodgkin’s Disease (IDHD), the Manchester Lymphoma Group index and, finally, the model of the International Prognostic Factor Project (IPPF). The proportion of the patient population identified by the low- or high-risk categories of each prognostic model was compared with the risk of failure actually observed in each category. Individual as well as joint performances of each prognostic index were univariately and multivariately analyzed in relation to OS, RFS and TTF by means of a proportional hazards model. None of the models identified a group containing > 10% of patients of the total population as having a failure risk of either < 10% or ≥ 50%. The systems of the IDHD, the MSK, and the IPPF showed the best prognostic power. Only these three models, when analyzed together, predicted clinical outcome with a statistically significant adequacy to the clinical data distribution of the study sample population. Integration of the three systems in a linear model dramatically improved their individual discriminatory capacity by identifying patients with 10 and 50% failure risks, respectively, in 23 and 24% of the study patient population, and in 19 and 25% of the test population. The program required for the calculations can be downloaded from the GISL web site at http://www.unimo.it/gisl/default.htm.
SB-C (p<0.0001), SNLG (p<0.0001), IDHD (p<0.0001) and IPFP (p<0.0004) models distinguish patient groups with significantly different 10-year expected OS; only PV-MO (p<0.0001), SB-C (p<0.0001), IDHD (p<0.0001) and IPFP (p<0.0002) models cluster patients in groups with different 5-year expected TTF. Besides, none of the models produced a group containing >10% of early or advanced stage as having a 5-year failure risk of either <12% or >50% respectively. For example 27/237 (11.3%) are the patients that have a number of prognostic factors (among those included in the IPFP model) higher than 3 as having a 10 years cumulative survival probability less than 50% and according to the IDHD model only 49/447 (11 %) are the patients that have an expected survival lower than 5 years. Moreover, we have cross-classified subgroups defined by individual indexes in order to verify the degree of association among them. Our analysis shows a degree of concordance between indexes of less than 20%. Multivariate Cox proportional hazard model demonstrated that none of the previous models offers by itself a meaningful interpretation of prognostic variability. Conclusions: At least in the GISSL data base none of the prognostic models proposed so far is reliable to define risk groups accurately, therefore we suggest caution in using them for treatment selection. We maintain that there is still room for improvement in prognostic evaluation in Hodgkin's disease even if we assume that it could not be obtained by simply adding further independent prognostic variables.

COO27
RISK OF SECOND SOLID TUMORS IN 1524 HODGKIN'S DISEASE PATIENTS CONSECUTIVELY TREATED AT FLORENCE UNIVERSITY HOSPITAL

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The prognosis of Hodgkin's disease (HD) patients has improved dramatically and the resulting long term survival of these patients has allowed the observation of late complications. We have evaluated the risk of second solid tumors (SSTs) in a series of HD patients consecutively treated between 1960 and 1991. All patients were followed since the date of HD diagnosis to 31/12/1995. Information on SST was collected by medical records during clinical follow-up carried out for these patients. In addition all death certificates in which any type of cancer other than HD was mentioned were also identified. For each potential case, the site, morphology and date of cancer incidence were verified using all available sources. Observed SST cases were compared with expected cases estimated on the basis of five-year age group, sex and calendar-year specific incidence rates provided by the Tuscany Cancer Registry of the province of Florence since 1985. Standardized Incidence Ratios (SIRs) (observed/expected cases) were estimated overall and for all specific cancer sites and 95% confidence intervals (CI) were calculated. Overall, 1,524 patients were included in the study. Median follow-up time was 8.4 years. For 13.7% of patients follow-up was 20 years or longer. An approximately two fold increased incidence of SST was evident in the cohort (100 cases observed versus 55 expected). Significantly increased risks were observed in males for nose (SIR 21.4; 95% CI 2.4-77.4) and lung cancer (SIR 3.4; 95% CI 2.2-5.1) and in females for breast cancer (2.0; 95% CI 1.2-3.4) and soft tissue sarcomas (SIR 11.79; 95% CI 1.3-42.5). Incidence of non-Hodgkin's lymphoma was also significantly increased in both sexes. For breast cancer the highest risk was evident in patients treated only with radiotherapy. Lung cancer risk was increased in patients treated with radiotherapy alone and with radiotherapy and chemotherapy combined. The risk of developing a SST tended to increase slowly throughout the follow-up. For lung cancer, in particular, the excess rose with increasing follow-up time from 2.7 after 9 years to 5.1 after 20 or more years of follow-up. No excess risk was found for breast cancer in the first 9 years after HD diagnosis, but afterwards a three fold increased risk was evident. According to age, the highest risk for breast cancer was evident in females treated before 30 years of age (SIR 4.4; 95% CI 1.8-9.0). A higher risk of developing lung cancer was also evident in both males and females treated before the age of 30 years.
Inherited deficiency of G6PD is a common and usually mild hemolytic disorder. However, some patients with rare G6PD mutations suffer from chronic non-spherocytic hemolytic anemia associated, in some case, with susceptibility to infections due to impairment of granulocyte function. This form of glucose-6-phosphate dehydrogenase (G6PD) deficiency is in principle, an excellent candidate for gene therapy by gene transfer into human hematopoietic cells (HSC) because (1) it is a severe lifelong condition for which there is no definitive treatment, (2) clinical manifestations are limited to blood cells. Lentiviral vectors (LV) are emerging as powerful tools for gene transfer into HSC because of their ability of transducing resting cells. We have constructed a LV vector pseudotyped with vesicular stomatitis virus G glycoprotein in which the transcription of hG6PD cDNA is driven by the CMV promoter which the transcription of hG6PD cDNA is driven by the CMV promoter. This vector has been used to transduce lineage negative cord blood cells in serum-free medium (MOI ~25) on retronectin-coated plates with several transduction conditions: (1) 5 hrs with or without cytokines; (2) 12 hrs of pre-culture followed by one to three transduction cycles of 12 hrs with cytokines. The transduced cells were (a) plated for hematopoietic colony forming cells (CFC) to test committed progenitor cells; (b) infected in a significant proportion of committed progenitors. Pre-culture and cytokines increased from less than 1% to up to 50% the percentage of human CFC expressing the transgene from NOD/SCID mice 8 weeks after transplantation. In three mice a high proportion of CFC (64%) expressing the transgene was still present up to 4.5 months after transplantation; (3) both mature red blood cells and white blood cells express a level of the transferred G6PD similar to that of the endogenous G6PD, a level that is expected to be therapeutically effective for severe G6PD deficiency.

In this study, by taking advantage of the fact that the G6PD-A transgene has a different electrophoretic mobility from the endogenous wild type G6PD B, we have been able instead to obtain internally controlled quantitative data on the expression of a natural gene. By this approach we have observed that in both erythroid and myeloid colonies the level of expression of the transferred G6PD driven by the CMV promoter was in most cases at least as high as that of the endogenous G6PD. In summary, (1) the primitive human HSC that are able to engraft into NOD/SCID mice need priming to be effectively transduced by LV vectors; (2) a high percentage of transduced CFC is detectable up to 4.5 months after transplantation; (3) both mature red blood cells and white blood cells express a level of the transferred G6PD similar to that of the endogenous G6PD, a level that is expected to be therapeutically effective for severe G6PD deficiency.
Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy in humans and is characterized by a wide biochemical and molecular heterogeneity. Up to date, more than 400 biochemical variants have been described worldwide, grouped into 5 classes on the basis of residual enzyme activity and clinical manifestations. By contrast, only 130 mutations and 7 with other sporadic molecular variants. The aim of this study was to identify the molecular defect in the 30 negative for common polymorphic variants previously analyzed for Mediterranean polymorphic mutations. A total of 237 G6PD-deficient unrelated subjects (226 Italian, 11 from other Mediterranean countries; 181 males, 56 females) have been previously analyzed for Mediterranean polymorphic mutations. We found 200/237 samples (84%) with one of the common mutations and 7 with other sporadic molecular variants. The aim of this study was to identify the molecular defect in the 30 negative samples. The thirty subjects (24 males, 6 females) were tested for mutations in G6PD gene by PCR-SSCP followed by direct automated sequencing. We identified 9 (30%) G6PD Chatham mutations (1003 G→A, 2 (6.7%) G6PD Santamaria (542 A→T, 376 A→G), 2 (6.7%) G6PD Guadalajara (1159 C→T) and single cases with G6PD Sibari, found in one heterozygous female with intermediate G6PD activity, is a known class III variant. This work, confirming the high molecular heterogeneity of G6PD defects, has also defined G6PD Chatham as a polymorphic variant (all. freq. = 3.4%) in Italy, G6PD Guadalajara and Tokyo as common in class I and G6PD Abruzzo as new in class III.

CO030
MOLECULAR CHARACTERIZATION OF G6PD-DEFICIENT MEDITERRANEAN SUBJECTS NEGATIVE FOR COMMON POLYMORPHIC VARIANTS
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The prenatal diagnosis of genetically determined disease is currently performed by the study of fetal cells isolated with invasive methods such as amniocentesis, funicolocentesis and villocentesis. Numerous Authors have tried to replace these methods of prenatal diagnosis with others non-invasive methods, with the purpose to not incur risks that the invasive procedures carry (increase abortive risk, maternal infections). In the last years the transplacental traffic of cellular elements of fetal origin has been shown and the use of these cells as potential elements for non-invasive prenatal diagnosis has been hypothesized. These elements are represented by erythroblasts, fetal lymphocytes and placental trophoblasts. The best cell for fetal identification and for molecular diagnostics has been revealed to be the fetal erythroblast. Fetal erythroblasts are rare in the maternal circulation, but their presence seems to be more substantial and constant between the VII and the XXV week of pregnancy. The erythroblast has peculiar characteristics among which the brief half-life in the maternal circulation, that excludes the risk of misdiagnosis (differently for instance from fetal lymphocytes, that can also survive in the maternal circle for 5 years, and therefore can also be found again in circulation in following pregnancies). The purpose of this study is to separate erythroblasts from maternal peripheral blood of women pregnant with thalassemic trait in the first trimester of pregnancy. Six pregnant women at 11 weeks have been enrolled. Peripheral venous blood (20 cc) separation of the erythroblasts was done through methodic magneting activated cell sorting (MACS) using monoclonal antibodies hapten conjugated anti CD45 (FITC) and monoclonal antibodies hapten conjugated anti CD71 (PE). The obtained results with the use of the double MACS (CD45 and CD71) have underlined an average purity on the recovered CD71 positive cellular population of 89.3% (range 88.3-90.8%). The overall average absolute number of CD71 positive nucleate erythroid elements recovered was 308 elements (range 132-490). The data have been obtained submitting the cellular suspensions, after the double MACS, to FACS analysis. For the evaluation of possible pollution of the CD71 positive population, FACS analysis was done with CD41 (PE) and showed the absence of CD41+ events confirming the data on the purity of the CD71 positive population previously reported. We concluded that the way of isolating erythroblasts and their following fetal identification constitute one of the main

CO031
SEPARATION OF ERYTHROBLASTS FROM PERIPHERAL BLOOD OF β THALASSEMIC WOMEN AT 11TH WEEK OF PREGNANCY BY MAGNETIC ACTIVATED CELL SORTING (M.A.C.S.)
G.C. Del Vecchio,* D. Campanale, L. Piccience,* M. Capocasale, R. Cammarota, D. De Mattia,* N. Tannia
and determinant point for advances of the studies of non-invasive prenatal diagnosis.


CO032
HEREDITARY SPHEROCYTOSIS: 160 CASES

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Hereditary spherocytosis (HS) is a very heterogeneous hemolytic disease, caused by a defect of the red blood cell membrane proteins, namely spectrin, ankyrin, band 3 or protein 4.2. The diagnosis is sometimes difficult in asymptomatic and mild forms. Splenectomy is usually effective. We present the clinical, hematologic and molecular characteristics of 160 patients (77 M and 83 F) with dominant (118) or not dominant (42) HS.

### Table: Hematologic and Molecular Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n=160)</th>
<th>Not splenectomized (n=134)</th>
<th>Splenectomized (n=26)</th>
</tr>
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<tr>
<td>Age (yr)*</td>
<td>170-80</td>
<td>17 (8-80)</td>
<td>24 (1-68)</td>
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<tr>
<td>Splenomegaly(°)</td>
<td>113/159 (71%)</td>
<td>89/133 (67%)</td>
<td>24/26 (92%)</td>
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<tr>
<td>Anemia(°)</td>
<td>63/159 (40%)</td>
<td>60/133 (45%)</td>
<td>3/26 (12%)</td>
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<td>Cholelithiasis(°)</td>
<td>39/160 (24%)</td>
<td>31/134 (23%)</td>
<td>8/26 (31%)</td>
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<tr>
<td>Neo-jaundice(°)</td>
<td>16/160 (10%)</td>
<td>13/134 (10%)</td>
<td>3/26 (12%)</td>
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<tr>
<td>Exchange-trans(°)</td>
<td>11/160 (7%)</td>
<td>8/134 (6%)</td>
<td>3/26 (12%)</td>
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<tr>
<td>Blood transf(°)</td>
<td>45/160 (28%)</td>
<td>34/134 (25%)</td>
<td>11/26 (42%)</td>
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<tr>
<td>Hb (g/dL) *</td>
<td>12.5 (6.0-18.4)</td>
<td>12.1 (6.0-16.9)</td>
<td>15.6 (9.2-28.4)</td>
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<tr>
<td>MCV (fl) *</td>
<td>87 (68-112)</td>
<td>86 (68-112)</td>
<td>90 (76-110)</td>
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<tr>
<td>MCHC (g/dL) *</td>
<td>35.2 (26.4-39.9)</td>
<td>35.2 (26.4-39.9)</td>
<td>35.2 (30.2-38.8)</td>
</tr>
<tr>
<td>Retics&lt;1010*</td>
<td>224 (37-909)</td>
<td>251 (38-909)</td>
<td>83 (37-439)</td>
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<td>Reticular (%)</td>
<td>5 (0-45)</td>
<td>5 (0-45)</td>
<td>9 (0-35)</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>1.5 (0.2-2.7)</td>
<td>1.6 (0.4-2.7)</td>
<td>0.7 (0.2-3.9)</td>
</tr>
<tr>
<td>ALT/AST (%)</td>
<td>143/148 (97%)</td>
<td>110/124 (96%)</td>
<td>24/24 (100%)</td>
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<tr>
<td>SDSPAGE (%)</td>
<td>122/160 (76%)</td>
<td>98/134 (73%)</td>
<td>24/26 (92%)</td>
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<tr>
<td>-SP</td>
<td>44/160 (27%)</td>
<td>34/134 (25%)</td>
<td>10/26 (38%)</td>
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<td>-AK</td>
<td>13/160 (8%)</td>
<td>9/134 (7%)</td>
<td>4/26 (16%)</td>
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<td>-B3</td>
<td>52/160 (33%)</td>
<td>42/134 (31%)</td>
<td>10/26 (38%)</td>
</tr>
<tr>
<td>-4.2-</td>
<td>13/160 (8%)</td>
<td>13/134 (10%)</td>
<td>0/26 (0%)</td>
</tr>
<tr>
<td>Undetected</td>
<td>30/160 (24%)</td>
<td>36/134 (27%)</td>
<td>2/26 (8%)</td>
</tr>
</tbody>
</table>

* Medians and ranges and (°) Nr of positive/total cases are indicated.

There were not significant differences among the various types of molecular defect, although subjects carrying spectrin and/or ankyrin deficiency seem to have a more severe phenotype (defined by Hb < 8 g/dL and/or multiple transfusions and/or splenectomy after the diagnosis).

**CO033**
TREATMENT OF SEVERE APLASTIC ANEMIA WITH ANTILYMPHOCYTE GLOBULIN, CYCLOSPORIN AND GRANULOCYTE COLONY STIMULATING FACTOR 5 m VERSUS 10 m/KG: A GITMO-EBMT RANDOMIZED STUDY

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Several studies reported a high probability for severe aplastic anemia (SAA) patients of becoming transfusion independent and of surviving after treatment with antilymphocyte globulin (ALG), cyclosporin (CyA) and granulocyte-colony stimulating factor (G-CSF), with a significant effect of neutrophil counts on outcome. Several problems are still unsolved, such as absent or incomplete response, relapse and CyA dependence. The aim of the present study was to compare overall results at 120 days from treatment, quality of hematological recovery and survival in a series of consecutive patients with SAA randomized to receive the same immunosuppressive protocol but different dosage of G-CSF (5 m versus 10 m/kg). Seventy-seven patients entered the study: 38 received G-CSF 5 m/kg/day; day +1 day +30 and 39 10 m/kg/day; day +1 day +30. All patients then received G-CSF 5 m/kg/day/day +31, day +90. The two groups were comparable for age, gender, degree of severity, and interval between diagnosis and treatment. Results at 120 days in 5 m/kg/day versus 10 m/kg/day treatment groups: complete/partial response was obtained in 26/38 vs 16/39 (p = 0.13); no response was observed in 7/38 and 11/39 (p = 0.29); 5/38 and in 12/39 died (p = 0.10) and in 12 (4 in the 5 m/kg/day group and 8 in the 10 m/kg/day group) follow-up was shorter than 120 days. WBC median peak values were 12.310 /dL and 11.650. Revaluation at the end of follow-up (10-1565, mean 473 days in the 5 m/kg/day or 10 m/kg/day group) follow-up was shorter than 120 days. WBC median peak values were 12.310 /dL and 11.650. Revaluation at the end of follow-up (10-1565, mean 536, and 16-1411, mean 473 days in the 5 m/kg/day or 10 m/kg/day therapy groups): 5/38 and 4/39 relapsed (p = 0.50); 6/38 and 12/39 died (p = 0.16). Interestingly, 3-year actuarial survival was significantly better in patients treated with lower dose G-CSF (81% vs 64%, p = 0.04). We conclude that higher G-CSF dosage does not seem to improve either survival or quality of hematological recovery.
B-cell neoplasms form a heterogeneous group of disorders derived from the clonal expansion of B-cells at different stages of maturation. The pathogenesis of B-cell neoplasia involves activation of proto-oncogenes and disruption of tumor suppressor genes. Aberrant promoter hypermethylation is an acquired epigenetic alteration, alternative to genetic lesions, causing inappropriate gene silencing. Promoter hypermethylation of the glutathione S-transferase p1 (GSTp1) gene has been recently reported in several human cancers. The GSTp1 gene encodes an enzyme implicated in the detoxification of a wide range of xenobiotics and chemotherapeutic agents. In particular, GSTp1 catalyzes the conjugation of glutathione with electrophilic compounds, resulting in less toxic and more readily excreted metabolites. Consistent with its role in defending normal cells against carcinogens, loss of GSTp1 activity causes an increased risk of cancer. On this basis, the aim of our study was to evaluate the involvement of GSTp1 promoter hypermethylation throughout the spectrum of B-cell neoplasia. The tumor panel was formed by 222 B-cell tumors representative of the clinico-pathologic spectrum of the disease in immunocompetent hosts, and 38 AIDS-related non-Hodgkin's lymphomas (AIDS-NHL). GSTp1 methylation status was analyzed by methylation-specific PCR of the gene promoter CpG island. Overall, GSTp1 hypermethylation occurred in 31/12 (25%) pre-B-cell acute leukemias, in 78/210 (37%) mature B-cell neoplasms. The tumor panel was formed by 222 B-cell tumors representative of the clinico-pathologic spectrum of the disease in immunocompetent hosts, and 38 AIDS-related non-Hodgkin's lymphomas (AIDS-NHL). GSTp1 methylation status was analyzed by methylation-specific PCR of the gene promoter CpG island. Overall, GSTp1 hypermethylation occurred in 31/12 (25%) pre-B-cell acute leukemias, in 78/210 (37%) mature B-cell neoplasms of the immunocompetent host, and in 18/38 (47%) AIDS-NHL. With respect to mature B-cell neoplasia, the frequency of GSTp1 hypermethylation varied markedly in different clinico-pathologic categories of the disease. GSTp1 hypermethylation occurred frequently in aggressive lymphomas, including sporadic Burkitt's lymphoma (13/25; 52%) and B-diffuse large cell lymphoma (B-DLCL; 36/92; 39%). In particular, GSTp1 promoter hypermethylation occurred throughout the clinico-pathologic spectrum of de novo B-DLCL (24/57; 42%), primary splenic B-DLCL (4/9; 44%), primary mediastinal B-DLCL (3/10; 30%), CD5+ B-DLCL (2/5; 40%) and primary central nervous system lymphoma (2/3; 67%). Among indolent lymphoproliferative disorders, a high frequency of GSTp1 hypermethylation was restricted to follicular lymphoma (10/18; 55%) and to hairy cell leukemia (6/8; 75%). Conversely, among other indolent lymphoproliferative disorders, GSTp1 hypermethylation was restricted to a minority of mantle cell lymphoma (2/9; 22%) and B-cell chronic lymphocytic leukemia (1/10; 10%), and was consistently absent in lymphoplasmocytoid lymphoma (0/7). In low grade MALT lymphoma, GSTp1 hypermethylation varied according to the disease site, since it was frequent in gastrointestinal MALT lymphoma (5/11; 45%), whereas it was absent in parotid MALT lymphoma (0/10). Finally, GSTp1 hypermethylation was consistently absent in multiple myeloma and plasma-cell leukemia (0/10). Among AIDS-NHL, GSTp1 hypermethylation occurred throughout the clinico-pathologic spectrum of the disease, including Burkitt's lymphoma (5/10; 50%), B-DLCL (8/11; 73%), primary effusion lymphoma (3/9; 33%) and Burkitt-like lymphoma (2/8; 25%). These data suggest that GSTp1 promoter hypermethylation is frequently implicated in B-cell neoplasia and may represent a major event in the pathogenesis of some, though not all, B-cell tumors. Moreover, because GSTp1 expression affects tumor cell resistance to genotoxic effects of alkylating agents and of anthracyclines and may influence prognosis of some human solid cancers, our results prompt investigations aimed at defining the prognostic value of GSTp1 hypermethylation in B-cell neoplasia.

The frequency of secondary acute leukemia (sAL) and myelodysplastic syndrome (sMDS) is increasing as a consequence of successful therapy for primary malignancies. Extreme variation in the frequency of microsatellite instability (MSI), the hallmark of DNA mismatch repair (MMR) deficiency, has been reported in these hematologic disorders. We are examining MSI and hMLH1 promoter methylation in a panel of Italian cases of sAL and sMDS in order to investigate: the frequency of MMR defects, the mechanism by which repair genes are inactivated and which therapeutic regimes are particularly associated with MMR inactivation. DNA was extracted from mononuclear bone marrow cells collected at diagnosis from 23 patients (18 sAML, 2 sMDS, 3 sALL) most of whom had received previous therapy for Hodgkin's or non-Hodgkin's lymphoma or breast carcinoma. Microsatellites BAT26, BAT25, D2S123, D17S250, D18S561 were analyzed by PCR. Samples with alterations at BAT26 were considered to be MSI+. Where normal DNA was available, instability was examined at the other loci. Methylation of the hMLH1 promoter was examined by PCR following digestion with either HpaII or MspI. Cases MSI+ were subsequently tested for mutations in the 8 poly-G tract present in the coding region of the proapoptotic gene BAX using direct sequencing. Fourteen of 22 DNA samples from which BAT26 could be amplified were MSI+. In the 8 cases for which normal DNA was available, instability was confirmed at additional loci. One case in which BAT26 was not amplifiable was unstable at 2 other loci (BAT25 and D2S123). Evidence of hMLH1 promoter methylation was obtained for two samples, both of which were MSI+. The hMLH1 promoter was unmethylated in three other MSI+ cases. All (4/4) secondary malignancies from Hodgkin's or non-Hodgkin's lymphoma...
patients treated with a methylating agent (procarbazine or dacarbazine) were MS1+. No mutations in the exonic repeat of the BAX gene were found in MS1+ cases. In conclusion inactivation of MMR is common among sAL and sMDS which may be MS1+ in > 60% of cases. Methylation of the hMLH1 promoter does occur but is not the only mechanism of MMR inactivation. MS1+ sAL and sMDS are particularly - but not exclusively - associated with the use of methylating agents.

MOLECULAR SCREENING

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In ethnic groups of Celtic descent, hereditary hemochromatosis (HH) is associated with homozygosity for C282Y HFE gene mutation or with compound C282Y/H63D heterozygosity. The occurrence of a HH phenotype in the absence of typical genotypes has suggested the existence of different genetic forms of the disease. One thousand and fifty potential blood donors were prospectively screened for HH in Northern Italy by transferrin saturation, serum ferritin, C282Y and H63D HFE mutations. Pedigree analysis showed no evidence of linkage of H63D mutation with increased transferrin saturation (cumulative lod score –2.41) and in relatives of probands with wild type/wild type genotype a recessive non HLA-linked locus genetic disorders.

CD30 IS INVOLVED IN REGULATING CXCR4/CXCL12 SYSTEM BIOLOGICAL ACTIVITIES

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The TNFR family molecule CD30 is expressed by activated and memory T cells depending on IL-4 stimulation preferentially in association with Th0/2-type responses. It mediates pleiotropic, mostly inhibitory effects. Arguing that CD30+ cells have peculiar tissue redistribution in disease, we demonstrate here, in the human Hodgkin-derived cell line L540, an established model for studying CD30 signaling, that CD30 regulates the prototypic lymphoid chemokine receptor CXCR4, which plays relevant roles in many organ systems and is a coreceptor for HIV-1 entry. Stimulation of L540 cells with antiCD30 agonistic antibodies led to accumulation of CXCR4 mRNA, which reached a plateau after 4 hours and did not require protein synthesis. Following mRNA transcription, membrane expression of CXCR4 in L540 cells increased as early as 12 hours, reached a plateau after 24 hours (MFI±SD: 839±122 vs basal 168±28, p<0.01) and was still increased after 5 days, enabling enhanced sensitivity to chemotactic activity of CXCR4-ligand CXCL12 (CI±SD: 10±2 vs basal 5±2, p<0.01). CD30 crosslinking also induced release of CCL5 and CCL3, up-regulation of the specific membrane-binding capacity for CCL3 and CCL4 and decreased proliferative activity. These findings delineate a new regulatory role for CD30, which may be relevant for T cell maturation, effector responses and in promoting cancer biology.

ERYTHROID KRÜPPEL-LIKE FACTOR EXPRESSION IN NORMAL AND MALIGNANT MEGAKARYOCYTES

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Erythroid Krüppel-like factor (EKLF) is an erythroid-specific member of the Krüppel family of zinc-finger-containing transcription factors. EKLF binds to the β-globin promoter CACC box and plays a critical role in adult β-globin gene expression. Target disruption of the mouse EKLF gene or mutation of its binding site is associated with β-thalassemia. It has recently been observed that adult mice with EKLF transgene have reduced platelet counts, suggesting that EKLF levels affect the balance between the megakaryocytic and erythroid lineages. Therefore, there might be a link between EKLF and megakaryocytopenia. However, the expression of EKLF in normal and malignant human megakaryocytes has not yet been studied in depth. Essential thrombocytopenia (ET) is a chronic myeloproliferative disorder whose behavior is governed principally by the abnormal proliferation of a malignant megakaryocytic clone. The molecular lesions which underlie the malignant clone are unknown. We investigated EKLF expression in CD34-derived normal (5 cases) and malignant megakaryocytes from ET patients (8 cases) and
in megakaryoblastic cell lines (MK1, MK2, HEL, B1647, M07e), using real-time RT-PCR to detect EKLF transcripts. Primary CD34+ cells taken from the bone marrow of ET patients and healthy donors were induced to differentiate along the megakaryocytic lineage in liquid suspension culture by continuous addition of 100 ng/mL thrombopoietin. After megakaryocytic cell purification using immunomagnetic beads, EKLF mRNA was quantitated by using real-time RT-PCR. Megakaryocytic cells purified from cell lines were also tested for EKLF expression. In order to minimize variability in the results due to differences in RT efficiency and/or RNA integrity among the unknown samples, the GAPDH house-keeping gene was also tested. Normalized levels of unknown samples were calculated as the ratios between EKLF and GAPDH. Megakaryocytic cells purified from cell lines were also tested for EKLF expression. In order to minimize variability in the results due to differences in RT efficiency and/or RNA integrity among the unknown samples, the GAPDH house-keeping gene was also tested. Normalized levels of unknown samples were calculated as the ratios between EKLF and GAPDH. Real-time RT-PCR showed that EKLF was significantly reduced in ET megakaryocytes (EKLF/GAPDH median: 0.0003 [0.0001-0.0007]) with respect to the normal counterpart (EKLF/GAPDH median: 0.001 [0.001-0.002]) (p<0.05). Megakaryoblastic cell lines showed variable EKLF/GAPDH ratios (MK1: 0.0003; MK2: 0.006; HEL: 0.0009; B1647:0.02; M07e: 0.001) and the median value was not significantly different from that of normal megakaryocytic cells. This study demonstrates that the transcription factor EKLF is expressed in normal megakaryocytic cells. It also suggests that its expression is impaired in malignant human megakaryocytes. We do not know whether the alteration in EKLF expression contributes to the genesis of the disorder or if it is secondary to malignancy. Intriguingly, however, several lines of evidence suggest that erythroid and megakaryocytic lineages arise from a common cell. Both lineages coexpress transcription factors like GATA-1 and NF-E2, as well as the erythropoietin receptor and the thrombopoietin receptor. Therefore, EKLF downregulation in ET megakaryocytes could alter the balance between the two lineages, giving advantages to megakaryocytopoiesis. Further studies are needed to identify EKLF target genes in the course of megakaryocyte development.

CO039 EFFECT OF ERYTHROPOIETIN AND INTERLEUKIN-3 ON TRANSCRIPTION FACTOR PHOSPHORYLATION IN GROWTH FACTOR-DEPENDENT HEMATOPOIETIC MULTIPOTENT PROGENITORS INITIATING ERYTHROID DIFFERENTIATION

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We previously reported T-antigen immortalized mouse multipotent hematopoietic progenitors which are maintained in the presence of erythropoietin (Epo) (Cairns et al. 1994 EMBO J. 13: 4577). From the original cells we recently derived a number of lines which grow in response to stem cell factor (SCF), and respond to interleukin-3 (IL-3) or Epo treatment by initiating differentiation and hemoglobinization. The cells can be grown reversibly in each of these factors, repressing or inducing hemoglobin mRNA synthesis, depending on SCF or, alternatively, in IL-3 or Epo. The locus control region (LCR) adjacent to the beta-globin cluster is already open (i.e. hypersensitive to DNase treatment) in nuclei of cells grown in SCF, and its DNase sensitivity is not modified by Epo or IL-3 treatment. However, Epo or IL-3 treatment induces beta-globin mRNA synthesis within 24 hours, in a process that is dependent on p38, but not MAPK activation. Withdrawal of Epo or IL-3 and replacement with SCF leads to decay of globin mRNA levels, and inhibition of p38 greatly accelerates the disappearance of beta-globin mRNA, indicating that p38 is required also for beta-globin mRNA maintenance. In these cells SCF, Epo and IL-3 activate the p38 and MAPK pathways; in collaboration with Dr. Tariq Enver (ICRF, London) we also showed that the erythroid transcription factor GATA-1 is phosphorylated in response to these factors, via a MAPK-dependent pathway. In contrast, STAT 5 is activated in response to Epo or IL-3, but not SCF.

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non-Hodgkin's lymphomas

CO040
THE CLINICAL SPECTRUM OF CUTANEOUS B-CELL LYMPHOMAS: POOR PROGNOSIS FOR PATIENTS WITH DIFFUSE LARGE CELL LYMPHOMA OF THE LEGS

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Skin is after the gastrointestinal tract the most frequent localization of extranodal non-Hodgkin's lymphoma. The majority of primary cutaneous lymphomas other than mycosis fungoides and Sézary syndrome have B-immunophenotype. The prognostic parameters of primary cutaneous B-cell lymphoma (CBCL) are still controversial. The aim of the present retrospective international multicenter study is to identify clinical features of prognostic significance for patients with CBCL. This analysis included 149 patients, 81 males and 68 females, with a median age of 56 years (range, 23 to 93 years). According to EORTC classification 72 patients (48%) had PCFCCL, 56 (38%) PCI and 21 (14%) diffuse large B-cell lymphoma (LBCL) of the legs. One hundred-thirty (87%) patients had stage IE, 19 (13%) stage IIE. In the majority of cases LBCL was diagnosed in trunk/arms (53%), while in 24% in head/neck and in 18% in the legs; 5% of patients had a generalized (>1 site) disease. Tumor was > 4 cm in 31% of cases and more than two lesions were recorded in 31%. The prevailing type of lesion was nodules (64%), while a minority of patients (8%) showed tumors. Few patients (10%) had B symptoms or elevated LDH. About 20% of patients were treated with surgery (n=19), chemotherapy (n=26) or radiotherapy (n=32) alone, while 70 cases (47%) received combined modality treatment. One hundred and thirty-six patients (91%) achieved complete remission, 7 a partial remission and 3 (2%) with LBCL of the legs were resistant to therapy. Among 143 responders, 47 (33%) eventually relapsed in the skin and 11 (24%) in extracutaneous sites. After a median follow-up of 40 months (range 1 to 209 months), 5-year estimate of overall survival (OS), event-free survival (EFS) and disease-free survival (DFS) were 84%, 59%, 62% respectively. By univariate analysis age (>65 years), histology (LBCL of the legs vs PCFCCL and PCI), number of lesions (>2), type of lesion (nodules vs plaques), site of lesion (leg vs head-neck and trunk-arms) and elevated LDH predicted a poor EFS. However, by Cox multivariate analysis, histology (LBCL of the legs) and type of lesions (nodules) were the only independent variables predictive of EFS. This retrospective international study shows that patients with LBCL belong to different risk categories requiring new therapeutic strategies in the setting of prospective multicenter studies.

CO041
TWELVE YEAR FOLLOW-UP OF 384 LYMPHOMA PATIENTS TREATED WITH HIGH-DOSE SEQUENTIAL CHEMOTHERAPY: PROLONGED EVENT-FREE SURVIVAL IN GERMINAL CENTER-DERIVED SUBTYPES


Several studies suggest that myeloablative regimes are most effective when delivered after maximal cytoreduction. Based on this concept, the high-dose (hd) sequential chemotherapy regimen (HDS) was designed, including an initial hd-phase, followed by circulating progenitor cell autograft. Since 1989 we have managed high-risk lymphoma (NHL) patients with the HDS approach. Following the initial experience with the original HDS in diffuse large cell lymphoma (DLCL), two second generation schemes were designed with two aims: a) to further increase the effectiveness in poor prognosis high-grade NHL (Ara-C-HDS); b) to develop a suitable schedule for patients with indolent lymphomas (i-HDS). So far, 184 patients (142 at disease onset, 42 refractory/relapsed) have been treated and are evaluable. All patients had advanced-stage disease, most of them presenting with adverse prognostic features. Median age was 47 years. Histologic subtypes were: 30 follicle-center (FCL), 24 marginal zone (MZL) or small lymphocytic (SLL), 14 mantle-cell (MCL), 8 T-AILD, 92 B-DLCL (18 with histological transformation), 7 CD30+ and 9 high-grade T-cell NHL. Overall, there were 8 toxic deaths (TRM =4,3%); secondary myelodysplasia/leukemia developed in 5 pts. (3%). 3 more patients developed a solid cancer. Long-term outcome was definitely better in B-cell (n=160) compared to non-B-cell NHL (n=24): the 12-year overall survival (OS) projection was 53% in the B-cell subgroup whereas the 9-year OS projection was 28% in non-B-cell NHL (median survival: 2.2 years). The 10-year OS of 123 B-cell NHL treated at disease onset was projected to 63%. In this latter group, a significant difference was observed in the long-term outcome between germinal-center derived (FCL, DLCL and transformed) subtypes (n=101) and MZL, SLL and MCL subtypes (n=22). In fact, the 10-year OS projection was similar in these two subgroups (68% and 59%, respectively). However, the event-free survival (EFS) curve was significantly better in germinal-center derived (52% projection at 11 years) compared to MZL-SLL MCL subtypes (13% projection at 10 years). In conclusion, the HDS approach: i. had acceptable toxicity and lower incidence of secondary cancer compared to other reported series of autografted NHL; ii. was not of benefit in non-B-cell subtypes; iii. allowed a prolonged survival in B-cell subtypes; iv. was associated with prolonged EFS in germinal-center-derived subtypes treated upfront, with expected 52% chances of cure, in spite of poor prognostic features. The most recent progress in the treatment of NHL was the introduction of monoclonal antibodies, in particular the anti-CD20 rituximab. In the newly developed third-generation HDS regimen, rituximab has been introduced in the HDS schedule (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 for both low-grade and high-grade NHL patients, presenting with unfavorable prognostic parameters. In conclu-
sion, our analysis shows that HDS: (i). is a feasible chemotherapy approach; (ii). is effective in high-risk B-cell lymphoma; (iii). can be successfully combined with novel therapeutic tools now available for lymphoma patients.

CO042
ANTHRACYCLINE-CONTAINING REGIMENS FOR THE TREATMENT OF FOLLICULAR LYMPHOMA: A RETROSPECTIVE ANALYSIS OF THE INTERGRUPPO ITALIANO LINFOMI

Department of Hematology Florence on behalf of Intergruppo Italiano Lинфomi

Patients with follicular lymphoma often have a long survival in spite of their frequent relapses. We report a retrospective analysis of a large series of patients with histologically confirmed diagnosis of follicular lymphoma treated in various Italian institutions. The Intergruppo Italiano Lинфomi (IIL) promoted a wide collection of patients with a diagnosis of follicular lymphoma between 1985 and 1996; 1096 patients were merged into a single working file of which 633 patients were treated with an anthracycline-polychemotherapy-containing regimen and a selected group of 128 patients treated without anthracyclines (CO). In the group who received anthracyclines 50% were male, 17% presented systemic symptoms, 75% were in advanced stages, 53% had bone marrow involved at diagnosis. According to IIL score 60% of patients were low risk, 25% intermediate risk and 15% high risk; using the IPI score 80% were low risk, 15% low-intermediate risk, 4% intermediate-high risk and 1% high risk. In the group treated with COP 40% were male, 13% presented systemic symptoms, 79% presented advanced stage, 56% had a positive bone marrow biopsy. The two groups were comparable for the major clinical characteristics except for age and in particular no differences were observed according to IPI index and IIL index. The complete remission rate (CR) for patients treated with anthracyclines was 69.2% and overall response rate was 92.5%. In comparison, in the group treated with COP, the rates were respectively 67.5% and 85.4%. After a median follow-up of 51 months, the 5 and 10-year overall survival was 80% and 66%. The 5 year overall survival (OS) for patients with partial remission (PR) was 65%. Disease-free survival (DFS) and Failure-free survival (FFS) at 5 years were respectively 61% and 56%. We have observed a better OS in patients treated with anthracyclines; 80% in comparison to 65% of patients treated without anthracyclines (p = 0004). Also patients treated with anthracyclines with partial response (PR) showed a better survival in comparison to patients with PR treated without anthracyclines, 65% vs 41% (p = 002). No differences were observed in DFS and FFS of the two groups. According to IIL prognostic index patients with low or intermediate risk showed a better OS if treated with anthracyclines (respectively p = 0001 and p = 0.0009), but patients with high risk did not show a statistically significant difference. Our retrospective analysis shows a significantly better survival in patients treated at diagnosis with anthracycline-containing regimens both in patients who obtained a CR or with PR. High risk patients according to the IIL model have a very poor prognosis and even though the use of anthracyclines can increase OS, probably this subset of patients should be proposed for more intensive therapy.

CO043
VACOP-B vs VHACOP-B+ HIGH-DOSE SEQUENTIAL THERAPY FOR AGGRESSIVE NON-HODGKIN’S LYMPHOMA. INTERIM ANALYSIS OF A PROSPECTIVE RANDOMIZED TRIAL

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In aggressive, advanced stage non-Hodgkin’s lymphoma (NHL), 2nd and 3rd generation regimens gave results similar to those observed with CHOP therapy. In 1997, the Milan Group suggested a statistical improvement in the outcome of pts treated with high-dose sequential therapy (HDS) in comparison with those pts treated with conventional chemotherapy (CT). Only B-cell type, G and H/WF, and BM negative pts were included. CR rate was 96% vs 70%, overall survival 81% vs 55%, and PFS 84% vs 49% in the two arms, respectively. In 1998, a randomized study of the NHLCSG compared CT + DHAP in case of persistent disease vs CT + high-dose therapy (HDT) with autologous bone marrow transplantation (ABMT) as front-line treatment for these pts. Pts with BM involvement were excluded. Results were similar in the two groups of pts. CR was 75% vs 73%, overall survival was 65% vs 65%, and PFS was 48% vs 60% in the two arms, respectively. We therefore started a new study in which pts with aggressive, advanced stage NHL were randomized to receive VACOP-B + HDS in case of persistent disease vs VACOP-B + HD (CY, 7 gr/m2; VP 16, 2 gr/m2 and BEAM + PBPC rescue). Aims: a) to confirm the Milan Group’s data; b) to evaluate possible use of HDS only when necessary. Two hundred and two patients were registered and 166 are now evaluable for response. First interim analysis shows 67% and 66% of CR respectively. With a median observation time of 26 months, actuarial curves show a 4-years probability of survival and of PFS of 61% and 46% respectively, with no difference between the two arms. When only B-cell type, G and H/WF NHL without BM involvement were censored, probability of survival and of PFS improved to 75% and 80%, and to 58% and 69%, respectively. Pts with T-cell type NHL and with BM involvement showed poorest results. When pts with BM involvement were excluded, the probability of survival and PFS were 73% and 72%, and 58% and 69% in the two arms respectively. This study seems to confirm the Milan Group’s data in a selected group of pts and suggests that results achieved with CT + HDS are similar to those observed with CT + ABMT. There is no apparent difference in using HDS after CT in all cases or only in the case of persistent disease.

CO044
IMPORTANCE OF GALLIUM SCAN RESTAGING FOR CURATIVE TREATMENT OF MEDIASTINAL LYMPHOMAS

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For mediastinal lymphoma patients, gallium-67-citrate single photon emission computed tomography (GaSPECT) provides unique information on the presence of residual active disease. We provide an updated report on a large cohort of patients whose management following induction therapy was based on
CO045
ADVANTAGES OF POSITRON EMISSION TOMOGRAPHY WITH RESPECT TO COMPUTED TOMOGRAPHY IN THE FOLLOW UP OF LYMPHOMA PATIENTS WITH ABDOMINAL PRESENTATION

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For abdominal lymphoma patients, fluoridine-18-fluorodeoxyglucose positron emission tomography (PET) provides unique information on the presence of residual active disease. We provide an update on the largest reported cohort of patients whose management followed tomography (CT) restaging. Fifty-nine patients with HD or aggressive NHL presenting abdominal involvement (35% with bulky disease) were studied with both PET and CT following combined chemotherapy/radiation treatment. After treatment 3/5 (60%) patients who were GaSPECT+/CT- relapsed, as compared with only 2/15 (13%) patients in the GaSPECT+/CT- subset. Among the 42 patients who were CT+, 2 of the 7 (28%) who were also GaSPECT+ relapsed, as compared with none of the 35 who were GaSPECT. The 6-year actuarial relapse-free survival rates were: 71% and 100% in the GaSPECT+/CT+ and GaSPECT+/CT- subsets respectively (p=0.003) and 48% and 93% in the GaSPECT+/CT+ and GaSPECT-/CT- subsets respectively (p=0.017). GaSPECT restaging is very valuable for initiation of appropriate second-line therapy for patients with residual active mediastinal disease and should be made widely available.

CO046
PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS: TREATMENT WITH HIGH-DOSE METHOTREXATE AND CYTARABINE FOLLOWED BY RADIOTHERAPY

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The prognosis of primary central nervous system lymphomas (PCNSL) is poor and their optimal treatment still controversial, although the use of drugs crossing the brain-blood barrier is likely to improve the outcome. This phase II study evaluated the efficacy and tolerability of a chemotherapeutic regimen consisting of high-dose methotrexate (MTX) and cytarabine (ARA-C) followed by radiotherapy (RT) in immunocompetent patients with PCNSL. We treated 28 unselected HIV-negative patients affected by PCNSL, aged 34 to 73 years (median 57). Histological diagnosis (in all cases of diffuse large cell lymphoma) was made by stereotactic biopsy in 18 patients and by surgery in 10. Order neurological performance status was 3-4 in 44% of patients. The following cycles of chemotherapy (CT) were given every 3 weeks: MTX 1g/m² iv over 24 hours (d1) with leucovorin rescue followed by ARA-C 2g/m² iv every 12 hours for 4 doses (d2-3). In the 14 patients aged less than 60 the doses of MTX and ARA-C were escalated to 2 g/m² and 3 g/m² respectively. After CT, whole brain irradiation (mainly 30 Gy, plus 10 Gy boost in case of solitary lesion) was given. In patients not achieving CR or near- CR (nCR, i.e. response >90%) after the 1st cycle, 3 cycles of CT were planned (2 cycles in the CR or nCR patients). Median follow-up is 29 months. Four patients, all aged over 60, were given only 1 cycle of CT due to toxic death (2) or severe infectious complications (2). 13 received 2 cycles, 9 received 3 cycles. Of the 28 patients, 27 are evaluable for response. CR or nCR was achieved in 20/27 (74%), including the 2 patients who died of toxicity, who were in CR and nCR at autopsy. So far 11 patients relapsed, 3 in sites other than the CNS (breast, liver, skin). Overall, 15 patients are alive. Hematological toxicity (grade 4 neutropenia and thrombocytopenia) was of short duration (median 3 and 3 days, respectively). Neutropenic fever occurred in about 40% of cycles, lasting mainly 2-3 days. In conclusion: this intensive CT regimen obtained a high rate of CR or nCR and appeared feasible in a group of unselected patients (including about 1/3 of cases >65 years). The occurrence of frequent relapses suggests that consolidation or maintenance with different drugs is indicated.
normal controls). At least 5 cases of large–cell HCV+NHL were from 0 to 8% (a prevalence comparable with 5.3% found in 466 lipherative disorders implications and justify the term tory findings, which may have significant biological and clinical

56% of patients projected to be alive at 6 years). However, HCV+ chemotherapy (52 vs 57%, p<0.05). Liver biopsy, performed in 48 patients, showed 26 cases of chronic (active or persistent) hepatitis (54.1%), 15 of cirrhosis (31.2%), 4 lymphomas (8.3%, 2 of whom with hepatic primitive disease), 1 liver carcinoma (2%), while in only 2 cases hepatic histology was normal. The majority of patients received frontline chemotherapies similar to those employed in comparable HCV-NHL. In 2 HCV+ patients hepatic function worsened and viral replication increased during the treatment. A comparison between HCV-NHL and a cohort of HCV-NHL patients matched for age, histology and treatments revealed that there was no difference in response rate to initial chemotherapy (52 vs 57%, p = n.s.) between these two groups. There was also no significant difference in overall survival (47 vs 56% of patients projected to be alive at 6 years). However, HCV+ subjects with significant ALT increase displayed a worse prognosis, showing a median survival of only 37 months. We conclude that HCV-NHL show some distinctive clinical and laboratory findings, which may have significant biological and clinical implications and justify the term HCV correlated lymphoproliferative disorders we propose to identify this specific subset of patients.

Daunoxome (D) is a combination of the anti-neoplastic agent daunorubicin with a unilamellar liposomal carrier system. Preliminary studies have indicated that liposomes < 100 nm in diameter accumulate preferentially within tumor tissue and therefore minimize normal tissue distribution. Other studies also suggest that daunoxome has antitumor activity in patients with NHL previously treated with anthracyclines. Vinorelbine (V) is a vinca alkaloid semi-synthetic derivative with high liposolubility and favorable toxicity profile and has shown a positive activity in relapsed Hodgkin’s and non-Hodgkin’s lymphomas. Despite curative potential, up to 50% of the patients with aggressive non-Hodgkin’s lymphoma relapse or fail front-line therapy. For those who cannot tolerated high dose or intensive therapy an effective and well tolerated regimen is warranted. From March 2000 to March 2001 fourteen pts. with relapsed resistant aggressive NHL and four elderly subjects not eligible for anthracycline-based chemotherapy were enrolled in a phase II study to evaluate the toxicity and safety of a combination of daunoxome and vinorelbine (DV). Eligible patients had resistant or relapsed NHL age > 16 years, no HIV or other serious infection, no CNS disease, normal renal and hepatic function and an EF > 50% and informed consent. Patients older than 70 years considered unsuitable for a standard dose CHOP regimen were also considered eligible. After informed consent patients received daunoxome 100 mg/m² in 100 ml of 5% dextrose over two hours and vinorelbine 25 mg m² i.v. bolus q 21 days. Eighteen patients were registered/enrolled and 16 are already evaluable for response. Median age is 65.3 years; 9 are male. Histology was diffuse large B-cell in 15 and mantle cell in 4. Clinical stage was I-II (6) III-IV (12). Serum LDH was elevated in 6. Nine patients had received anthracycline before DV and 8 pts were refractory to the regimen immediately preceding DV. Response was seen in 8 out 14 of relapsed resistant pts (3 CR + 5 PR 57%) and in all 3 elderly therapy naive pts, evaluable for response (3 CR). The hematological and non-hematological toxicity was negligible besides pain and phlebitis at the site of the vinorelbine infusion. Alopecia and mucositis were notably absent. There were no cardiac adverse events and in addi-

CO048 LIPOSOMAL DAUNORUBICIN AND VINORELBINE IN RELAPSED NON-HODGKIN’S LYMPHOMA; EARLY RESULTS

Introduction: Indolent lymphomas usually have a high clini-
cal response rate with conventional treatment, but molecular
remissions are rare and relapses occur frequently. Fludarabine-
containing regimens induce good clinical response but no data are yet available regarding molecular remissions. The combina-
tion of fludarabine-containing regimens and rituximab may improve clinical and molecular response, but few data are avail-
able regarding their feasibility, safety and possible immunosup-
pressive effects. Patients and methods. From March 1999 to December 2000, 24 patients with advanced stage relapsed fol-
licular non Hodgkin’s lymphoma (NHL) or indolent NHL (at diag-

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for the presence of IgH/BCL2 rearrangement and/or FR2-JH clonality was performed on lymph-node samples or bone marrow (BM) at the beginning of the treatment and on BM after FND and after rituximab. Results. Median age was 60 years (range 50-75); 12 males and 12 females; 13 follicular NHL, 4 lymphocytic NHL, 5 marginal NHL, 2 immunocytoma; 12 patients were treated at relapse (6 =1 relapse); 23 had stage III/IV disease, 16 BM involvement, 9 bulky disease and 4 had >1 extranodal sites. Up to date, 16 patients are evaluable for feasibility, safety and clinical response. 15/16 patients completed the therapeutic plan and one patient did not receive rituximab due to progression of disease after FND. Treatment has been entirely performed in an outpatient setting. No toxic deaths were observed. Severe toxicity (WHO Grade 3-4) was: neutropenia 6 cases after FND and 1 after rituximab; one bacterial infection after FND; 1 AMI after FND and 1 DVT during FND chemotherapy. Clinical response to treatment was evaluated after FND and after rituximab. Clinical response to 4 FND was as follows: complete remission (CR) 6 patients, complete remission unconfirmed (CRu) 3, partial remission (PR) 6, non response (NR) 1. Clinical response to rituximab, at the end of the whole treatment program, was as follows: CR 12, CRu 1, PR 2, NR 1. With addition of rituximab, complete response (CR + CRu) increased from 56% to 81%. In 12/16 patients a molecular marker of disease was detectable at the beginning of the treatment: a PCR-negative status was achieved in 4/12 patients after FND chemotherapy, while molecular remission was observed in 8/12 patients after Rituximab treatment. All these 8 patients were in clinical CR. Conclusions: FND chemotherapy followed by Rituximab has been shown feasible in an outpatient setting with low toxicity, but neutropenia (29%) was observed 7/25 failures (progression or relapse) and 2/30 early deaths. In conclusion the association of GM-CSF-CHOP-RTX is characterized by a very high response-rate also in heavily pretreated patients and, even though the incidence of hematological and extra-hematological toxicities is not negligible, this approach seems feasible and should also be tested earlier in patients who have not been pretreated.
(84%) pts, of whom 63/73 (86%) relapsed and 6/9 (66%) refractory, achieved a PR/CR after IEV chemotherapy and were considered eligible for intensification with HAC-ASCT. Five of 69 eligible pts did not receive HAC-ASCT (one for refusal and four for low performance status). After a median follow-up of 42 months (6-114) the 3-yr OS and EFS for all 82 pts were 40% and 24%, respectively. Two/64 (3%) transplant-related deaths were observed. The 3-yr OS and EFS were, respectively, 45% vs 0% (p = 0.007) and 27% vs 0% (p = 0.02) for the chemoresistant vs compared to chemoresistant pts. For pts with ER or LR the OS were 40% vs 52% (p = 0.12) and the EFS were 20% vs 34% (p = 0.04). The 3-yr OS was shorter (19% vs 58% p = 0.005) and the EFS was 9% vs 33% (p = 0.002) in pts with an high IPI at relapse. Conclusions: IEV has been an active and well tolerated salvage regimen able to induce a clinical response and to mobilize PBSC in a high rate of pts. Among chemoresistant relapses, pts with an early relapse (<12 months from diagnosis) and a high-risk IPI (2-3) at relapse are a group of pts at poor prognosis for whom new strategies may be investigated.

COO52
HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS TRANSPLANTATION vs INTENSIFIED CHEMOTHERAPY MegaCEOP IN POOR PROGNOSIS DIFFUSE LARGE CELL LYMPHOMA: COMPARABLE REMISSION RATE AND TOXICITY IN A MULTICENTER RANDOMIZED TRIAL BY ITALIAN LYMPHOMA INTERGROUP


On the behalf of Italian Lymphoma Intergroup (ILI)

Introduction. Poor prognosis diffuse large cell lymphoma (DLCL) fared poorly with standard chemotherapy with CR and FFS < 50% and 30%. High dose chemotherapy (HDC) was reported to give better results compared to standard chemotherapy in randomized studies. Promising results were reported with intensified chemotherapy with G-CSF. No data are yet available comparing these two approaches. A multicenter study was performed by the Italian Lymphoma Intergroup (ILI) to compare feasibility, toxicity and outcome of HDC regimen with autologous stem cell transplantation (ASCT) vs an outpatient intensified chemotherapy regimen (MegaCEOP). Patients and methods. From January 1996 to September 2000, 131 pts < 60 years with DLCL with intermediate-high IPI score were considered eligible for intensification with HDC-ASCT. Five of 69 eligible pts did not receive HDC-ASCT (one for refusal and four for low performance status). After a median follow-up of 42 months (6-114) the 3-yr OS and EFS for all 82 pts were 40% and 24%, respectively. Two (MegaCEOP) due to sepsis. Two secondary ANLL and MDS were observed at 23 and 30 months after ASCT in group A. With a median follow-up of 30 months, DFS, OS and FFS rates at 2 years were respectively: group A 76%, 57% and 41% vs group B 67%, 54% and 44%. Conclusions. HDC+ASCT or an intensified outpatient chemotherapy are feasible and effective in a cooperative setting without severe toxicity. Toxic death rate is comparable to standard chemotherapy. Toxicities and CR rates are not different in the two treatment groups, but a longer follow-up is needed to show different relapse rates.

COO53
HIGH-DOSE SEQUENTIAL CHEMOTHERAPY IN POOR-RISK HIGH-GRADE B-CELL LYMPHOMA: IMPROVED RESULTS BY REGIMEN INTENSIFICATION WITH HD-ARA-C


Cure chances for diffuse large cell lymphoma (DLCL) patients with intermediate-high IPI score are poor. Their 5-yr survival is around 30-40% with conventional treatments, such as CHOP or MACOP-B. In order to improve long-term outcome, intensive treatments with autograft have been considered upfront. In a previous randomized study, we observed that high-dose sequential (HDS) chemotherapy and autograft offered better results in terms of CR achievement and CR duration compared to MACOP-B. That study was performed in a well-defined DLCL subset, with exclusion of patients with bone marrow disease or transformed or T-cell histologies. We subsequently evaluated HDS efficacy on 77 consecutive unslected poor-prognosis DLCL patients aged <65 yrs The original scheme (o-HDS) was employed in the initial 32 patients while a further intensified scheme, supplement- ed with a 6-day hd-Ara-C course (c-HDS), was employed in the following 45 patients. All patients had advanced-stage disease and high aaIPI score (2-3), with the exception of 3 patients with low aaIPI but unfavorable histology (2 transformed, 1 T-cell high-grade). Overall results were superior to those observed in conventionally-treated historical controls, with 7-yr overall survival (OS) and event-free survival (EFS) projections of 60% and 55%, respectively (median follow-up: 3 yrs). Good results were observed in the 68 B- DLCL with 62% and 57% OS and EFS projections, respectively. The outcome of B- DLCL patients treated
with either the original HDS or the C-HDS was then investigated. The 7-yr OS and EFS projections of o-HDS treated patients were 50% and 48% respectively, while the 4-yr OS and EFS projections of C-HDS treated patients were 72% and 67%, respectively. Differences in long-term outcome between these 2 treatment groups were statistically significant (p<0.05). The results indicate that: (i) the HDS approach offers higher chances of prolonged survival and event-free survival compared to conventional chemotherapy in aIPI 2-3 DLCL patients; (ii) the intensified C-HDS shows higher therapeutic efficacy compared to the original HDS; (iii) a long-term survival around 70% can be expected in poor-prognosis B-DLCL patients treated with C-HDS. Ongoing studies are addressed to verify whether a further improvement may be achieved in B-DLCL combining C-HDS with the anti-B cell rituximab monoclonal antibody.

C0054
"AGE-ADJUSTED" HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS WITH AGGRESSIVE NON HODGKIN'S LYMPHOMA: ADEQUATE PBPC YIELD, LOW TOXICITY AND FAVOURABLE PRELIMINARY RESULTS

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Introduction. Elderly patients (> 60 yrs) are usually considered not eligible for high-dose chemotherapy (HDC). However, most of them might tolerate ASCT with age-adjusted HDC regimens. Patients and methods. Patients ranged between 61 and 70 years with aggressive NHL in relapse/progression or at diagnosis with intermediate/high or high risk according to IPI and/or BM involvement were enrolled into this study. The therapeutic scheme includes three phases: A) 6 weeks of P-VEBEC chemotherapy in patients at diagnosis; A bis) 2 courses of DHAP (at 75% of the total dose) for patients in relapse/progression or with slow dose after P-VEBEC; B) intensification with a 2-day course of mitoxanthrone 8 mg/m² plus high-dose cytarabine (HD-ARAC) 1500 mg/m²/12hr plus dexamethasone 4 mg/m²/12hr and G-CSF 5 µ/kg/d from day +3 to harvest peripheral blood progenitor cells (PBPC); C) ASCT conditioned by BCNU 200 mg/m² d-6, ARAC 200 mg/m²/12hr plus VP-16 100 mg/m²/12hr dd -5 to -3, melphalan 120 mg/m² d -2, reinfusion of at least 5x10⁹/kg CD34+ cells, G-CSF 5µg/kg from day +1 to hematological engraftment. Results. Since January 1998, twenty-six pts have been enrolled: median age 65 years (range 61-70), 20 males, 6 females, 12 B-DLCL de novo, 8 transformed follicular, 3 mantle cell, 1 anaplastic and 2 peripheral T-cell NHL. Twelve pts were in relapse/progression: 9 in first early relapse (< 12 months), 1 in second, 1 in partial remission and 1 in progression; all pts showed advanced disease at relapse/progression; 10/12 pts were previously treated with anthracycline-containing regimens. Fourteen pts were treated at diagnosis: 9 were at intermediate/high or high risk according to IPI criteria, 10 had bone marrow involvement. Up to now, 24/26 are evaluable for clinical response and toxicity. An adequate PBPC yield (> 5x10⁹/kg) was obtained in 21/24 pts with a median of 13x10⁹/kg CD34+ cells (range 5.2-45). PBPC yield was not achievable in 3 pts: two were previously heavily treated with chlorambucil and the last one progressed while on therapy. Twenty of 24 pts were autografted. Four pts did not receive ASCT: two because of progressive disease and two due to inadequate PBPC yield. The median times to achieve neutrophils > 0.5x10⁹/L and platelets >50x10⁹/L were 7 days (range 4-9) and 18 days (range 2-79), respectively. No toxic deaths were observed. Grade 3 and 4 WHO toxicities were: mucositis in 2 patients and infections in 4 (2 bacterial, 1 pulmonary aspergillosis, 1 CMV infection). Clinical response in the 12 pts in relapse/progression was as follow: 6 CR, 1 PR, 5 NR. Among the 12 pts treated as first line therapy at diagnosis 10 patients achieved CR and 2 NR. Conclusions. These preliminary results suggest that a good PBPC yield is achievable after intensified chemotherapy with mitoxanthrone and HD-ARAC in elderly pts at diagnosis or pre-treated. An age-adjusted therapy with a modified HDC and BEAM regimen followed by ASCT is feasible and effective with low toxicity also in elderly patients with aggressive NHL.

C0055
DIFFUSE LARGE CELL LYMPHOMA IN ELDERLY PATIENTS TREATED WITH MiCEP PROTOCOL

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The increasing incidence of non-Hodgkin’s lymphoma (NHL) in elderly people led to the design of a specific regimen for these patients (pts). In 1989 in order to reduce organ toxicity we devised a novel chemotherapy scheme (MiCEP) utilizing drugs with limited organ specific toxicity: mitoxanthrone, cyclophosphamide, etoposide and prednisone. We presented the preliminary results of this protocol in Leukemia and Lymphoma in 1995. By 1996 we had collected 145 pts older than 65 years with a de novo diagnosis of large cell lymphoma. These patients were treated with the MiCEP protocol between 1989 and 1996. Their median age was 72.3 years (range 65-87), 78 pts were stage III; 40 were symptomatic, 38 had LDH value higher than normal and 18 had bulky disease. According to the IPI 45 were low-risk, 40 were low-intermediate risk, 39 were intermediate-high risk and 18 were high risk. Sixty three percent (91/145) achieved a complete remission (CR), 48 (33%) obtained a partial response with an overall response of 96%. With a median follow-up of 66 months (range 1-138 months) overall survival (OS) and progression-free survival (PFS) at 5 years were respectively 52% and 43%. In a multivariate analysis response to therapy was the only parameter statistically significant for overall survival (p = 0000). Of the 91 CRs, 24 (26%) relapsed, the majority in the first year of treatment and the disease-free survival (DFS) for CRs was 68% with a median follow-up of 53 months (range 2 - 116 months). The treatment was well tolerated; we observed five early deaths due to complication of therapy (3%). The hematologic toxicity was characterized by WHO grade 4 neutropenia in ten patients (6.8%) and grade 4 thrombocytopenia in two patients (1.3%). The extrahematologic toxicity was cardiologic grade 3 in one patient; alopecia was observed in about half the patients. 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 only parameter significantly related to overall survival.

CO056
P-VEBEC vs MiniCEOP. RESPONSE RATE, OVERALL, DISEASE-FREE AND EVENT-FREE SURVIVAL, AND EVALUATION OF QUALITY OF LIFE IN A RANDOMIZED TRIAL IN ELDERLY PATIENTS AFFECTED BY DIFFUSE LARGE CELL LYMPHOMA

On behalf of Italian Lymphoma Intergroup (ILI)

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Aim. In a multicenter randomized study conducted by the ILI we evaluated the feasibility of treatment, CR, OS, DFS and EFS rates and quality of life (using the EORTC QLQ-C30 questionnaire) of elderly patients treated with Mini-CEOP (Epi 50 mg/m², Cyclo 750 mg/m², Vbl 5 mg/m² day 1 and Pdn 50 mg/m² days 1-5 every 21 or 28 days) or P-VEBEC chemotherapy (a weekly regimen with Epi 50 mg/m², Cyclo 350 mg/m² and VP16 100 mg/m² on week 1,3,5,7; Vbl 5 mg/m² and Bleo 5 mg/m² on week 2,4,6,8; Pdn 50 mg/day p.o. in the first 2 weeks and thereafter every other day). The QOL questionnaire was administered at diagnosis, after 4 cycles of MiniCEOP and after 6 weeks of PVEBEC and one month after the end of the therapeutic program. Results. From June 1996 to December 1999, 284 patients affected by diffuse large cell lymphoma (DLCL) were enrolled into the study. Inclusion criteria were: age >65 years, stage II bulky, III or IV, PS ≤2 (ECOG); G-CSF was not routinely planned but allowed. 250 patients are evaluable for clinical characteristics, 37 pts were not eligible, 22 pts did not have complete data. Clinical characteristics are well balanced between the two groups Mini-CEOP vs P-VEBEC, namely age, sex, LDH, PS, bulky disease, IPI, co-morbidity, dose intensity of etoposide and cyclophosphamide. Also the main severe toxicities were similar: 53% of pts achieved CR, 58% with Mini-CEOP and 47% with PVEBEC. PR were recorded in 13% vs 35% (p = 0.0005). NR or PD were observed in 21% vs 12%. TD was 8% vs 6%. With a median follow-up of 30 months OS and EFS were respectively 41% and 41% for both groups of pts; DFS was 54% vs 39% (p=n.s.). In a multivariate analysis the only prognostic factor for OS was IPI and that for EFS was LDH. The QOL questionnaire was completed for only 91 patients. The data obtained showed that there was an improvement of life condition comparing baseline values evaluated before therapy with those evaluated at the end of treatment: pain (p=0.01), sleep (p<0.015), unger (p<0.01) and global health status (p<0.03). Conclusions. Our study shows a comparable low toxicity for both regimens, a better response rate for miniCEOP, comparable OS, EFS and DFS rates. This study outlined the importance of evaluating QOL in planning an appropriate therapy for elderly patients. New therapeutic approaches are probably warranted to improve the outcome of such a group of patients.

CO057
POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS: AN EPIDEMIOLOGICAL AND CLINICAL STUDY FROM A SINGLE CENTER: UPDATE

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Solid organ allograft recipients present high risk for developing neoplasia because of a chronic immunocompromised status. Post-transplant lymphoproliferative disorders (PTLD) are the most frequent neoplasia after skin and lip cancer. The overall prevalence is 0.5-10%. At our Hospital 38 cases of PTLD were diagnosed in 2265 solid organ transplant (Tx) recipients from 1973 to December 2000 (1.67%). According to what reported in clinical literature, the prevalence varies in the different types of organ transplants: 3.8% heart Tx, 0.9% kidney Tx, 1% liver Tx, 1.6% lung Tx. The median onset is 53 months after transplant (range 1-174). The clinical, histological, and main characteristics are reported in the table.

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<tr>
<th>Time of onset</th>
<th>EBV correlation</th>
<th>Histology</th>
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<tr>
<td>Early (≤12 months)</td>
<td>Late (&gt;12 months)</td>
<td>Early Late EBV+ EBV- PH* PLD§ ML^</td>
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<td>%</td>
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<td>N°Pts.</td>
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*PH: plasmocitic hyperplasia; PLD: polymorphic lymphoproliferative disorders; ML: Malignant Lymphoma/Plasmocytoma like.

In 8 (21%) patients (pts) the diagnosis of PTLD was made at autopsy. Three pts were treated in their own city hospitals, in 3 pts no therapeutic treatment was performed (except the reduction of immunosuppressive regimens) because of their severe clinical conditions and they died between 1 and 10 days from diagnosis. Only 22 pts received chemotherapy tailored on clinical and histological characteristics (chemotherapy + surgery + radiotherapy + antiviral + high dose immunoglobulin + interferon), and it is possible to evaluate the efficacy of treatment. Seven out 22 (31.8%) died of progressive disease plus infection after a median of 74 days (range 18-131); 15/22 (68%) got complete remission (CR). Out of these 15, one died of unrelated causes, one is cured for disease relapse and 13 are still alive at 690 days (median) from diagnosis (range 210-1515). PTLD is a severe complication in transplanted patients. Neither clinical nor histological criteria have been found to predict a response to disease treatment; even first line therapeutic regimens haven’t been defined yet.
MOLECULAR EVALUATION OF RITUXIMAB IN VIVO PURGING EFFECT IN HIGH-DOSE CHEMOTHERAPY TREATED INDOLENT LYMPHOMAS

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The aim of this study was to determine by the GeneScan PCR, as a sensitive molecular method, whether the anti-CD20 monoclonal antibody (rituximab) could modify the molecular status in patients affected by indolent lymphomas treated by high-dose therapy. Thirty-two patients (23 follicular lymphomas, 2 chronic lymphocytic leukemias, 4 lymphoplasmocytic and 3 mantle cell lymphomas) were included in the study. Eighteen patients started CHOP /rituximab/high-dose therapy as first line strategy and 14 at progression or relapse. Sixteen patients received 2 doses of the anti-CD20 monoclonal antibody before mobilization; 20 patients were transplanted and cases in the rituximab arm received 2 further antibody doses after the PBSCT. Thirty-one of 32 patients survived with a median follow-up of 32 months. The overall response rate was 75%, with higher CR rate (81% versus 69%) and lower progression/relapse rate (19% versus 31%) in the rituximab arm. A molecular marker was obtained for 18 patients at diagnosis (10 in the R-HD and 8 in the HD group). PCR-negativity rate of harvests resulted higher in the R-HD group than in the HD group (80% versus 31%). This advantage for patients treated with rituximab resulted also when the cut off for the analysis decreased (44% of PCR-negativity versus 19%). In the control cohort 5 cases PCR-negative before treatment harvested contaminated cells; no patients in the R-HD arm, negative at diagnosis, harvested PCR-positive precursors. In the same cohort one PCR-positive patient harvested PCR-negative cells only after the ex vivo purging. In the remaining cases, the CD34+ ex vivo purging did not significantly modify molecular status of leukapheresis products. Nevertheless, also PCR-positive harvests showed a reduction of the peak areas of 1-3 log, resulting in <1 neoplastic cell among 1000 cells in 14 cases. Twenty patients were autotransplanted and 14 were evaluated by molecular assays post-PBSC. 11 (79%) resulted PCR-negative. 3/5 cases PCR-positive at the start of therapy became negative after transplantation in the R-HD group and 2/3 in the HD cohort. All positive patients were PCR-positive at the start of HD therapy and received contaminated precursors. No patients receiving a PCR-negative graft resulted positive after PBSCT; only in the R-HD group did 2 patients. PCR-positive early after transplant achieve molecular remission after 3 months. So, the present study confirms the advantage of the rituximab in the achievement of either clinical or molecular remission in indolent NHL cases.

DIFFUSE LARGE B-CELL LYMPHOMA

CO039

PROMOTER HYPERMETHYLATION OF THE O6-METHYLGUANOSINE-DNA METHYLTRANSFERASE GENE IS A FAVORABLE PREDICTOR OF SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMA

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Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy, arising from germinal center B-cells and accounting for approximately 40% of all B-cell non-Hodgkin’s lymphomas (B-NHLs). DLBCL are markedly heterogeneous in terms of clinical course and treatment response. Although clinical indicators may define, at least in part, the prognosis of these patients, they continue to have different outcomes. Recently, it has been proposed that the tumor genotype may affect DLBCL prognosis. In this respect, the identification of new molecular prognostic markers may help to further stratify patients into different risk groups. O6-methylguanosine-DNA methyltransferase (MGMT) is a gene encoding a DNA repair enzyme that protects cells from the cytotoxic effect of alkylating agents and is a potential determinant of resistance to these drugs. In particular, absent MGMT expression favors the genotoxic damage exerted by alkylating agents, including cyclophosphamide. Inactivation of MGMT expression by promoter hypermethylation is the main mechanism for loss of MGMT activity in human cancer. A previous study showed that MGMT promoter hypermethylation is an important determinant of the response of gliomas to alkylating agents and may help to further stratify patients into different risk groups. O6-methylguanosine-DNA methyltransferase gene is a favorable predictor of survival in diffuse large B-cell lymphoma.
mechanism implicated in AIDS-related lymphoma: a novel pathogenetic mechanism implicated in AIDS-related lymphoma

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The process of somatic hypermutation primarily occurs in germinai center (GC) B-cells and is characterized by introduction of point mutations in the variable region of immunoglobulin (IgV) genes. Although the mechanisms underlying the mutation process are not fully understood, data indicate that the mutation activity is coupled to the transcription process and may target non-Ig sequences expressed in GC. It has been recently shown that BCL-6, a gene known to be involved in lymphomagenesis, is affected by a somatic hypermutation process in lymphomas derived from GC and post-GC B-cells. These findings raise the possibility that other genes, expressed in GC B-cells and involved in B-cell growth and differentiation, can be targeted and altered by the somatic hypermutation process. To address this issue, we performed a mutational analysis of different genes actively expressed in GC B-cells and known to be involved in lymphomagenesis. These include: i) PAX-5, encoding a transcription factor regulating late stages of B-cell differentiation; ii) TFF, encoding a member of the Ras superfamily of small GTPases; iii) PIM-1, a serine-threonine kinase controlling hematopoietic cell growth; and iv) c-MYC, a transcription activator and apoptosis modulator. For each gene, a region spanning the promoter and the translated exons, where some of the mutations are accumulated during GC transit of B-cells. Evidence of anti-germinal center (GC) stage of B-cell differentiation (BCL-6/CD138+) was observed in PTLD, as well as in B-cells. Evidence of antigen selection of IgV genes was analyzed by the binomial (Chang-Casal) and the multinomial statistical methods. PTLD utilized all of the most common IgV genes, with no specific bias for a given IgV family. Twenty of 22 (91%) PTLD were found to carry somatic mutations in IgVH and/or IgVL genes, at a fre-

CO060

NON-IMMUNOGLOBULIN GENE HYPERMUTATION: A NOVEL PATHOGENETIC MECHANISM IMPLICATED IN AIDS-RELATED LYMPHOMA

The findings of somatic hypermutation in IgVH or IgVL family. Twenty of 22 (91%) PTLD were found to carry somatic mutations in IgVH and/or IgVL genes, at a frequency of each gene was comparable among each category of the disease. Mutations were often multiple, heterogeneous, occasionally included small deletions, and revealed the presence of mutational hot spots. The mutation frequency in each individual case ranged from $0.59$ to $6 \times 10^{-6}/bp$. Analysis of the mutation pattern (predominance of single nucleotide substitutions, rare small deletions, excess of transitions over transversions and specific motif targeting) suggests a hypermutation mechanism with features similar to those affecting the IgV and BCL-6 sequences. In PIM-1 and c-MYC, the affected sequence also included translated exons, where some of the mutations led to aminoacid changes. The implications of these observations are twofold. First, mutations of c-MYC, PAX-5, PIM-1 and TFF are frequently implicated in AIDS-NHL and may represent an important event in the pathogenesis of the disease. In particular, mutations affecting the regulatory regions of these genes have been shown to be capable of altering gene transcription, at least in some cases. Second, the finding of somatic hypermutation in genes other than IgV sequences suggests that the infidelity of the Ig hypermutation machinery may underlie, at least in part, the genesis and progression of B-cell lymphomas, such as the AIDS-NHL, that are associated with the GC microenvironment.

CO061

MOLECULAR ANALYSIS OF IMMUNOGLOBULIN GENES IN POST-TRANSPLANT LYMOPHOPROLIFERATIVE DISORDERS: IMPLICATIONS FOR DISEASE PATHOGENESIS AND HISTOGENESIS

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Post-transplant lymphoproliferative disorders (PTLD) represent a heterogeneous group of lymphoproliferations arising in immunosuppressed transplant recipients. PTLD comprise a histologic spectrum ranging from polyclonal hyperplasia to frank lymphoma or multiple myeloma. The precise molecular events involved in PTLD pathogenesis are poorly understood and their histogenetic derivation has not been addressed in detail. In order to refine the pathogenesis and histogenesis of the disease, we investigated the usage, the mutation pattern and the antigen selection process of immunoglobulin variable (IgV) heavy (H) and light (L) chain genes in a panel of 24 PTLD. In parallel, other geneotypic and phenotypic markers of histogenesis were investigated and correlated to IgV mutations. These included BCL-6 and CD138 expression, which segregate the germinal center (GC) stage of B-cell differentiation (BCL-6+/CD138+) from later stages of maturation (BCL-6+/CD138+); and BCL-6 mutations, which are accumulated during GC transit of B-cells. Evidence of antigen selection of IgV genes was analyzed by the binomial (Chang-Casal) and the multinomial statistical methods. PTLD utilized all of the most common IgV genes, with no specific bias for a given IgV family. Twenty of 22 (91%) PTLD were found to carry somatic mutations in IgVH and/or IgVL genes, at a fre-
Introduction. Point mutations of the BCL-6 noncoding regions of the BCL-6 proto-oncogene are frequently detected in B diffuse large cell lymphoma (B-DLCL). Although BCL-6 mutations have been investigated extensively at the molecular level, a thorough analysis of the clinical correlation of these mutations has not been performed to date. Patients and methods. BCL-6 mutations were examined by DNA direct sequencing in samples of lymph nodes or bone marrow at diagnosis in 103 patients with B-DLCL; 76 received standard anthracycline-containing chemotherapy and 27 high dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT) up front. Results. BCL-6 point mutations were found in 53/103 (51%) patients, including 38/76 (50%) treated with standard chemotherapy and 15/27 (56%) treated with ASCT. The presence of BCL-6 mutations was correlated with outcome and with the clinical features at diagnosis (age, sex, stage, bone marrow involvement, symptoms, LDH level, bulky, IPI, n* extranodal sites). Patients with BCL-6 mutations had a significantly higher LDH level (66% vs 38%, p<0.05), and bulky disease (51% vs 32%, p=0.05). Among the 76 patients with B-DLCL treated with standard chemotherapy, BCL-6 mutations did not affect complete remission and overall survival. However, mutated patients showed a significantly improved disease-free survival (DFS) (85% vs 48%, p<0.05) and notably only four relapsed in less than 8 months, compared with those without BCL-6 mutations, who did so continuously up to 69 months. The multivariate regression analysis (p=0.01) with DFS as endpoint identified three prognostic factors: performance status, BCL-6 muta-

Background and objectives. TCR gene analysis using PCR is a highly specific and sensitive method for detecting neoplastic lymphoid clones in mycosis fungoides (MF) both at diagnosis and during treatment. The aim of the study was to investigate whether molecular analysis at the end of the combination therapy with PUVA + interferon (IFNα) in early MF can be valuable in predicting further clinical relapses. Design and methods. Twenty-one cases of early MF (7 st. IA, 10 st. IB, 4 st. IIA) were included for the study: all the patients received the same protocol of combination therapy with PUVA + IFNα and had skin biopsies both at diagnosis and at the end of therapy. PCR for TCRγ gene rearrangement was done using three primers matching Vγ I, II, III/IV segment families and two primers matching Jγ: and Jpγ junction segments on DNA extracted from formalin-fixed and paraffin-embedded tissue. Results. At diagnosis all the cases but one were PCR positive. At the end of therapy 6 cases showed molecular remission by PCR: all these patients had achieved clinical complete remission (CR) and are still free of disease after a median time of 32 months (range, 19-64 months). The remaining 15 cases, showing persistence of monoclonal T cell populations, included 2 patients with partial response to therapy; 11 patients who had achieved CR, but relapsed after a median time of 27 months (range, 3-46 months) and 2 patients in CR and disease-free after 20 and 29 months. Our data confirm the value of PCR at diagnosis as a complementary tool for histologic assessment of skin lesions. Moreover, PCR is very useful in predicting which patients will relapse after achieving CR with the combination therapy and possibly benefit from a maintenance therapy with low-dose IFNα to perform progression-free survival.

CO062
PROGNOSTIC SIGNIFICANCE OF MOLECULAR REMISSION AFTER PUVA PLUS IFNα IN EARLY STAGE MYCOSIS FUNGOIDES

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Background and objectives. TCR gene analysis using PCR is a highly specific and sensitive method for detecting neoplastic lymphoid clones in mycosis fungoides (MF) both at diagnosis and during treatment. The aim of the study was to investigate whether molecular analysis at the end of the combination therapy with PUVA + interferon (IFNα) in early MF can be valuable in predicting further clinical relapses. Design and methods. Twenty-one cases of early MF (7 st. IA, 10 st. IB, 4 st. IIA) were included for the study: all the patients received the same protocol of combination therapy with PUVA + IFNα and had skin biopsies both at diagnosis and at the end of therapy. PCR for TCRγ gene rearrangement was done using three primers matching Vγ I, II, III/IV segment families and two primers matching Jγ: and Jpγ junction segments on DNA extracted from formalin-fixed and paraffin-embedded tissue. Results. At diagnosis all the cases but one were PCR positive. At the end of therapy 6 cases showed molecular remission by PCR: all these patients had achieved clinical complete remission (CR) and are still free of disease after a median time of 32 months (range, 19-64 months). The remaining 15 cases, showing persistence of monoclonal T cell populations, included 2 patients with partial response to therapy; 11 patients who had achieved CR, but relapsed after a median time of 27 months (range, 3-46 months) and 2 patients in CR and disease-free after 20 and 29 months. Our data confirm the value of PCR at diagnosis as a complementary tool for histologic assessment of skin lesions. Moreover, PCR is very useful in predicting which patients will relapse after achieving CR with the combination therapy and possibly benefit from a maintenance therapy with low-dose IFNα in order to prolong progression-free survival.

CO063
THE PRESENCE OF POINT MUTATIONS OF THE BCL-6 GENE IS A FAVORABLE PROGNOSTIC FACTOR TO PREDICT A PROLONGED DISEASE-FREE-SURVIVAL IN B-DIFFUSE LARGE CELL LYMPHOMA

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Introduction. Point mutations of the BCL-6 proto-oncogene are frequently detected in B diffuse large cell lymphoma (B-DLCL). Although BCL-6 mutations have been investigated extensively at the molecular level, a thorough analysis of the clinical correlation of these mutations has not been performed to date. Patients and methods. BCL-6 mutations were examined by DNA direct sequencing in samples of lymph nodes or bone marrow at diagnosis in 103 patients with B-DLCL; 76 received standard anthracycline-containing chemotherapy and 27 high dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT) up front. Results. BCL-6 point mutations were found in 53/103 (51%) patients, including 38/76 (50%) treated with standard chemotherapy and 15/27 (56%) treated with ASCT. The presence of BCL-6 mutations was correlated with outcome and with the clinical features at diagnosis (age, sex, stage, bone marrow involvement, symptoms, LDH level, bulky, IPI, n* extranodal sites). Patients with BCL-6 mutations had a significantly higher LDH level (66% vs 38%, p<0.05), and bulky disease (51% vs 32%, p=0.05). Among the 76 patients with B-DLCL treated with standard chemotherapy, BCL-6 mutations did not affect complete remission and overall survival. However, mutated patients showed a significantly improved disease-free survival (DFS) (85% vs 48%, p<0.05) and notably only four relapsed in less than 8 months, compared with those without BCL-6 mutations, who did so continuously up to 69 months. The multivariate regression analysis (p=0.01) with DFS as endpoint identified three prognostic factors: performance status, BCL-6 muta-

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tions and number of extranodal sites, confirming the independent prognostic value of BCL-6 mutations. There was a trend for failure-free-survival to be better for patients with BCL-6 mutations (63% vs 30%, \(p=0.09\)). In the 27 patients treated with ASCT, BCL-6 mutations did not correlate with different outcome. Conclusions: These results suggest that the presence of BCL-6 mutations may be a favourable prognostic marker to predict a higher chance to be free of disease in B-DLCL treated with standard chemotherapy. However, given the small sample size and the retrospective nature of the study, these findings should be interpreted with caution and need to be confirmed in a larger series of patients.

A high-dose (hd) chemotherapy scheme was designed for the collection of peripheral blood progenitor cells (PBPC) after extensive chemotherapy-mediated tumor debulking. The scheme included the sequential administration of hd-cyclophosphamide (CY) (7 g/m²) and hd-Ara-C (2 g/m² b.i.d. for 6 consecutive days) and final myeloablative consolidation with PBPC autograft. PBPC harvests were scheduled following both hd-CY and hd-Ara-C. To minimize hematologic toxicity, small aliquots of PBPC (≤3 x10⁶ CD34+ cells/kg) collected following hd-CY were reinfused following hd-Ara-C. The treatment was delivered to 112 patients (median age: 43) with lymphoid malignancies (107 non-Hodgkin lymphoma, 4 Hodgkin’s lymphoma, 1 amyloidosis); 75 patients were at disease onset, whereas 37 had relapsed or refractory disease after first-line conventional therapy. PBPC mobilization was assessed in terms of peak values of circulating CD34+ cells/μL as well as total CD34+ cells/kg collected. In 61 patients CFU-GM/kg were also evaluated. At the time of maximal mobilization following hd-CY, 93 high mobilizer patients had >20 circulating CD34+ cells/μL whereas the remaining 19 low mobilizer patients did not reach this cut-off value. Within the high-mobilizer group, 88 patients received hd-Ara-C and 79 of them (90%) still showed high mobilization; overall, median collected CD34+ cells x10⁶/kg were 17.8 (range 3-94) and 19 (range 0-107), after hd-CY and hd-Ara-C respectively (p=ns). In spite of poor mobilization after hd-CY, 16 out of 19 low mobilizer patients could restore good harvests following hd-Ara-C; overall, median collected CD34+ cells x10⁶/kg were 1.4 (0-3.1) and 10.2 (0-37), after hd-CY and hd-Ara-C respectively (p=0.00007). A similar pattern was observed when PBPC were evaluated as CFU-GM/kg. Complete and durable hemopoietic reconstitution occurred following autograft with post-hd-Ara-C PBPC. Thus, the scheme: (i.) allowed to collection of large amounts of PBPC following two shortly-spaced hd-chemotherapy courses; (ii.) made it possible to perform adequate PBPC collections for autografting also in patients presenting with low mobilization capacity.
A prospective, randomized, multicenter clinical trial was started in Italy in 1996 with the aim to compare single (Tx-1; Arm A) vs. double (Tx-2; Arm B) transplantation of autologous peripheral blood stem cells (PBSC) as front line therapy for previously untreated patients with multiple myeloma (MM). Treatment plan in both arms included VAD × 4, collection of PBSC using HD-CTX (7 g/m²) and, upon recovery of hematopoiesis following Tx, maintenance therapy with INF-α. High-dose chemotherapy consisted of melphalan, 200 mg/m² (MEL), administered before both Tx-1 and the first of double Tx, and the combination of melphalan, 120 mg/m², and busulfan, 12 mg/kg, (Mel-Bus), administered before the second Tx. An interim analysis of the first 178 patients who entered the study was performed in December 2000 and results are herein reported with a median follow up of 30 months. The probability of completing the assigned treatment program was 80% for the 97 patients randomized to Arm A and 65% for the 81 patients randomized to Arm B. On an intent-to-treat basis, stringently defined complete remission (CR) rate was 22% for patients randomized toTx-1 and 26% for patients randomized to Tx-2. No statistically significant difference in the 4-year projected probability of survival was observed between the two groups using an intent-to-treat analysis (74% for Tx-1 vs. 71% for Tx-2). In contrast, Tx-2 conferred a significantly longer duration of disease control compared to Tx-1, both on an intent-to-treat basis (median, 31.5 vs. 20.5 months; p = 0.03) and whether the entire treatment program was actually received (median, 38 vs. 22.5 months; p = 0.01). As a result, median event-free survival of Tx-2 recipients was significantly longer than that of patients who actually underwent Tx-1 (39 months vs. 24.5 months; p = 0.04). This finding was confirmed by a landmark analysis at 5 months (p = 0.02) and a multivariate analysis that included application, or not, of Tx-2 as a time-dependent covariate (p = 0.008). Final analysis of the study is required to give definite conclusions concerning the benefits, if any, from double PBSC Tx as front line therapy for symptomatic patients with MM.
two groups, as were CD19+, CD3+ and CD8+ lymphocytes, while CD4+ and NK subsets were superior in PBSC, as was the CD4/CD8 ratio. By contrast, recovery of the cytotoxic CD8 subset, from the first to the twelfth month, was superior in BM as compared to PBSC. We have not observed any difference in incidence of opportunistic infections in the two groups, while there was a difference in relapse rate (PBSC: 66.6%; BM : 25%). The different kinetics of immunological recovery is probably related to both the number of reinfused cells and their source. It is difficult to explain the cytotoxic CD8 subset expansion in the BM group. It will be interesting to understand whether this population could be responsible for the better control of the leukemic disease in the BM group.

CO068
INFECTION MORBIDITY LATE AFTER AUTOLOGOUS CD34+ SELECTED PERIPHERAL STEM CELL TRANSPLANTATION
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Autologous CD34+ selected peripheral blood stem cell transplantation (PB SCT) has been recently applied in order to obtain an inoculum virtually devoid of tumor cell contamination. An increase of both morbidity and mortality after these procedures raised some concern on the safety of this strategy which has not been proved to be superior to conventional PB SCT. We studied 45 consecutive patients transplanted from November 1994 to January 2000 using CD34+ selected cells, among them 31 patients (group A) (median age 44 years, range 17-60y; 20 male, 11 female) affected by NHL (15), HD (6), MM (9), plasmacell leukemia (1), were free of malignant disease recurrence with a minimum follow-up of 1 year (29 months, range 12-72). These patients were evaluated for infectious complications occurring from day +31 to day +360 after transplantation. Data were compared to a group of 30 patients undergoing unmanipulated PB SCT, during the same period (group B), with a median follow-up after transplantation of 34.5 months (range 12-81). Patients received a median number of CD34+ cells of 3.8 (range 1.2-16) ×10^9/kg in group A and 10.7 (range 2-50.2) ×10^9/kg in group B. Selection system was Ceprate SC (Celipiro, Bothel, WA, USA) or CliniMACS (Miltenyi Biotech, Bergisch Gladbach, Germany). All patients were treated in a single bed with reverse isolation or single sterile rooms equipped with positive air pressure. All patients received oral ciprofloxacin as prophylaxis during neutropenia, prophylactic treatment for Pneumocystis Carinii pneumonia with trimethoprim-sulfamethoxazole, acyclovir until day +150 and an episode of pneumonia on day +150 and an episode of Varicella Zoster Virus infection on day +270 respectively in two patients undergoing CD34+ enriched PB SCT. No episode was recorded. Bone regeneration was faster as compared to historical surveillance and appropriate antimicrobial prophylaxis is pursued particularly in the early phase after transplant.
controls; these data need to be supported by larger studies under controlled conditions. The clinical use of ex vivo purified and expanded mesenchymal progenitors is particularly appealing.

CO070
HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL RESCUE IN HODGKIN’S DISEASE: THE EXPERIENCE OF THE ISTITUTO DI EMATOLOGIA ED ONCOLOGIA MEDICA “L. e A. SERÎGNOLI” DI BOLOGNA

Istituto di Ematologia e Oncologia Medica “L. e A. Seràgnoli”, Università di Bologna

Between September 1982 and December 2000, 97 patients (pts) with Hodgkin’s disease were autografted at Bologna University. There were 56 males and 41 females, their median age was 32 yrs (range 14-60), median interval from diagnosis was 29 months. 61 pts (63%) received bone marrow (BM) stem cells, 27 peripheral blood progenitor cells (PBSC), 8 liquid phase BM cells; 1 had combined BM and PBSC transplantation. Disease status at HDT was: 60 responsive disease (1st complete remission (CR) 1, 2nd CR 2, 3rd or subsequent CR 7; partial remission (PR) 10; responding relapse 26; untested relapse 14); 37 refractory disease (primary refractory 18; resistant relapse 19). HDT was BEAM in 84 pts, CVB in 8 pts, other conditioning regimen was: TBI 45%, mitoxanthrone 60 mg/m2 + Melphalan 160 mg/m2 (day+48), mitoxanthrone 60 mg/m2 + Melphalan 160 mg/m2 (day+48), followed by PBSC reinfusion. We studied the ovarian function of 30 women (NHL 19, HD 5, MM 6), median age 45 years (range 16-53), with a median follow-up of 40 months. Before treatment all patients had regular menses and many of them had had previous pregnancies. Results. All women became menopausal after this treatment. The ovarian function was evaluated with hormonal level (FSH, LH, estradiol, progesterone) after 3 months and then every 6 months from the end of therapy. In all cases the levels accorded with menopausal status and 23/30 pts had menopausal symptoms: 11/30 vasomotor instability, 6/30 vaginal dryness, 6/30 mood changes. In no cases did ovarian function recover: 27/30 women received hormone replacement therapy with symptoms improvement. Four of 30 interrupted hormonal therapy, but no regain of ovarian function was observed. Conclusions. Loss of ovarian function occurs in all women after allogeneic BMT and it results from damage to the ovaries by TBI-containing conditioning regimens. In our experience the use of two alkylating agents in a few weeks seems more toxic than TBI. This is to be considered when young women are enrolled in this kind of protocol.

CO072
DELAYED DOSE OF GLYCOSYLATED COLONY-STIMULATING FACTOR POST AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANT: PRELIMINARY RESULTS OF A RANDOMIZED TRIAL

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Although G-CSF utilization after PBSC transplantation determines a faster granulocytes recovery, not all the studies have shown clear advantages associated with its usage and hence the necessity to conduct sufficiently extensive randomized trials in order to clarify this subject. Since a delayed start of G-CSF has been shown to have a comparable efficacy in respect to G-CSF started at day +1, we choose to study this schedule. We have initiated a prospective randomized clinical trial comparing low dose (4 μg/kg/day) glycosylated G-CSF (Myelostim-Italfarma) starting from day +5 post-transplant, with a no treatment arm, in patients who underwent PBSC transplantation for LNH, HD, MM and solid tumors. Patients affected by acute leukemia were excluded from the study. The treatment was stopped for...
neutrophils count >500 for at least 2 days. Until now 21 patients have been enrolled in the study, 7 MM, 12 lymphoma and 2 solid tumors; 11 patients were randomized to receive G-CSF from day +5 and 10 patients were randomized in the no treatment arm. There were no significant differences in the two groups of patients regarding the characteristics of PBSC infused, age, state of diseases and type of the conditioning regimens used (HD-PAM, BEAM, TT-BUS-PAM). In the treatment group G-CSF was administered for an average of 5.9 days. Compared to the control group, patients treated with G-CSF showed shorter neutropenia (N<500) (Logrank \( p=0.001 \)) and a reduction in the need of parenteral nutrition (13 days in the G-CSF group vs. 16 days in the control group).

<table>
<thead>
<tr>
<th>G-CSF d +5</th>
<th>no G-CSF</th>
<th>p value</th>
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<tbody>
<tr>
<td>CNT infused ( \times 10^9 ) kg</td>
<td>4.35</td>
<td>11.19</td>
</tr>
<tr>
<td>CD34+ ( \times 10^9 ) kg infused</td>
<td>9.7</td>
<td>6.9</td>
</tr>
<tr>
<td>N&lt;500</td>
<td>+13</td>
<td>+14</td>
</tr>
<tr>
<td>PLT&gt;20000</td>
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<td>+14</td>
</tr>
<tr>
<td>PLT requirement (U)</td>
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<td>5 units</td>
</tr>
<tr>
<td>Discharge day</td>
<td>+19</td>
<td>+20</td>
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</tbody>
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No statistical differences were noticed for others clinical parameters, such as platelet recovery, platelet transfusion requirement, duration of hospital stay and fever episodes. The preliminary analysis of our data shows that the use of a low dose of G-CSF with a delayed beginning (from +5) is effective in the reduction of neutropenia post PBSC transplantation. The number of patients required in order to adequately evaluate whether administration of G-CSF is associated with clinical and economic advantages is about 40 patients per arm. The study is still ongoing.

Peripheral blood progenitor cells (PBPC) are increasingly used for autografting after high-dose chemotherapy in hematological and non-hematological malignancies. PBPC can be detected by the surface expression of CD34, and their quantification in peripheral blood (PB) and leukapheresis products (LP) can be obtained by a simple cytofluorimetric technique. An accurate evaluation of circulating CD34+ cells is crucial for the proper timing of PBPC collections. Moreover, the reproducibility of CD34 cells evaluation among different laboratories allows standardization of the minimum threshold dose of PBPC required to safely obtain engraftment. A Multicenter Quality Control Trial for CD34+ cell counts has been carried out by the Study Group for Immunological Markers. Seventeen laboratories have taken part in the trial. Between January 1998 and April 2001, a total of 472 specimens have been sent to the referring laboratory in 15 deliveries, each consisting of a sample of fresh blood and a sample of LP from each patient. All centers were requested to evaluate blood counts and CD34+ cell counts (percentage and absolute count) in both specimens using routine techniques as per single center guidelines. Results were collected and analyzed at the flowcytometry facility of the Hematology Department of Turin University and reported to the participating centers after each quality control delivery. Data from 464/472 (98%) specimens were evaluable for the study. Fifteen/17 laboratories (88%) used a dual-platform approach and the 2 remaining centers a single-platform assay. Thirty centers (76%) used the Milan protocol, whereas 4 have recently implemented the ISHAGE protocol during the study. To evaluate the interlaboratory variation in CD34+ cell counts, CV% was calculated for percentage and absolute CD34 counts, and for white blood cell counts in both PB and LP. Mean CV% for CD34+ % was 25.95 ± 14.31 in PB and 14.58 ± 5.66 in LP; mean CV% for CD34+/μL was 26.37 ± 14.32 in PB and 17.35 ± 6.38 in LP; mean CV% for WBC/μL 6.47 ± 3.18 in PB and 10.33 ± 5.68 in LP, respectively. Lower CV values were always observed from LP compared to PB specimens; this may be due to the lower content of CD34+ cells in PB that increases the probability of percentage error. The mean CV value we obtained for CD34% is significantly higher than that obtained (mean CV 14.3%) in a previous Quality Control Trial from our Group, in which standardized procedures and reagents were used. These findings are consistent with those obtained by Barnett et al. (Br J Hematol 2000; 108:784) who showed a reduction of interlaboratory variation in CD34+ cell counts using standardized protocols. This is the first Italian Quality Control Trial for CD34+ cell analysis by flowcytometry, and the only one employing fresh blood products instead of stabilized samples. Each participating center can adjust and improve its technique by comparing results from different facilities. Due to the clinical relevance of CD34...
evaluation, the use of the same standardized technique in a cooperative group is warranted.

Acknowledgments to V. Occhiena, Becton & Dickinson, Fresenius Companies for partial support of the Quality Control Trial.

CO074
TICARCILLIN-CLAVULANIC ACID PLUS AMIKACIN versus CEFTAZIDIME PLUS AMIKACIN AS EMPIRIC THERAPY FOR FEVER IN ACUTE LEUKEMIA: A RANDOMIZED STUDY

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Dissemination of β-lactamase producing organisms in neutropenic patients is an increasingly important issue; thus new antibiotic combinations with the adjunct of the β-lactamase inhibitor, should be tested. To evaluate the efficacy and tolerance of ticarcillin-clavulanic acid plus amikacin versus ceftazidime plus amikacin as empiric therapy in febrile patients with acute leukemia, a total of 127 evaluable episodes in 98 patients enrolled during the 98-2000 period, were examined. Patients were randomly treated with intravenous ticarcillin-clavulanic acid, 240 mg/kg/day, plus amikacin, 15 mg/kg/day (TCA group=62 febrile episodes) or with ceftazidime, 90 mg/kg/day, plus amikacin (CFA group = 65 febrile episodes). Overall success rate (survival through neutropenia) both without and with modifications (addition of a glycopeptide, antifungal or other antibiotics) was 93% for the TCA group and 92% for the CFA regimen. Success without modifications (afebrile at 72-96 hours) was 39% for TCA treatment and 31% for CFA; success with modifications was 55% and 61% respectively. Failure (death due to documented or presumed infection) was 7% for TCA therapy and 8% for CFA. In both regimens, success without modifications was significantly higher in fever of undetermined origin (FUO) than in clinically or microbiologically documented infections (DI): respectively 67% and 33% for TCA treatment, 75% and 25% for CFA. Success with modification was higher for TCA regimen in FUO than in DI (68% versus 32%), for CFA regimen higher in DI than in FUO (62% versus 38%), with a significant difference between the two groups. (p = 0.012)

Episodes with severe neutropenia (ANC<100/µL) were treated successfully without modifications in 54% for the TCA group and in 85% for the CFA group (p = 0.05).Tolerance was good in both regimens. Resistance rates of Gram negative bacteria responsible for bloodstream infections were identical: 64%. All strain-related deaths involved multi-resistant isolates. We conclude that the two regimens are both effective as initial treatment of febrile and prolonged neutropenia, although severe neutropenia was successful more frequently in the CFA group; in both regimens patients with DI are likely to require modified therapy, even if in TCA group modifications in FUO occurred more frequently than in CFA group.

CO075
CONTROL OF PSEUDOMONAS AERUGINOSA COLONIZATION AT HEMATOLOGY UNITS: EFFICACY OF PATIENT AND STAFF COHORTING

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The onset of multiple drug-resistant bacteria is a common event in hospitals and the control of these hospital-acquired infections remains a major challenge. Spread and persistence of resistant bacteria is often the consequence of transient contamination of the personnel’s hands, environmental contamination and excessive use of antibiotics. Control measures have relied on improved asepsis and handwashing, isolation of infected and colonized patients and antibiotic control. Here we describe the efficacy of the cohorting measure for the reduction of patients colonized with P. aeruginosa, which led to the control of an outbreak of multi-resistant P. aeruginosa. The study was conducted in the Hematology Department of the University of Catania, an 18-bed department. Before cohorting, two opposite hallways with three two-bed rooms in one side (left-side) and 6 two-bed rooms on the other side (right-side) composed the ward. A central sink was placed in the nursery between the hallways. There was no predefined selection for admission of patients. With the application of cohorting measures the two wards were separated by double doors, three sinks were placed in every hallway to allow easy hand washing facilities. The left-side of the ward was used to treat patients referred for the first time to the unit. After the first stay in the hospital, patients treated in the left-side continued to be treated in the left side. All the other patients were treated in the right-side of the ward. Two different nurses and medical staff were created without changing the patient-to-nurse ratio. We investigated the P. aeruginosa distribution and percent of patient colonization in our unit during the outbreak (July 98 - July 99), and after the application of the cohorting and the other control measures (Aug. 99 - July 2000) through surveillance and clinical cultures (7970 tests from 692 consecutive hospital admissions of 401 patients).

<table>
<thead>
<tr>
<th>Before cohorting</th>
<th>After cohorting</th>
<th>Total</th>
<th>Right side</th>
<th>Left side</th>
<th>New pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/98-7/99</td>
<td>8/99-7/00</td>
<td>8/99-7/00</td>
<td>8/99-7/00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N.of bacteriological tests</td>
<td>4155</td>
<td>3815</td>
<td>2747</td>
<td>1068</td>
<td></td>
</tr>
<tr>
<td>N. of documented P. Aeruginosa isolation</td>
<td>196</td>
<td>71</td>
<td>62</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>N. of patients colonized with P. Aeruginosa</td>
<td>45</td>
<td>33</td>
<td>27</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Percent of pts colonized with P. Aeruginosa</td>
<td>23.9%</td>
<td>15.4%</td>
<td>18.6%</td>
<td>8.8%</td>
<td></td>
</tr>
<tr>
<td>N. of patients</td>
<td>188</td>
<td>213</td>
<td>145</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>N. of hospital admissions</td>
<td>340</td>
<td>352</td>
<td>250</td>
<td>102</td>
<td></td>
</tr>
</tbody>
</table>

Our data show a reduction of the percent of patients colonized with P. aeruginosa after the application of cohorting and other
control measures. The reduction from 23.9% of the pre-cohorting to 18.6% (p=0.3 Fisher test) obtained on the right side of the ward is mainly related to the improved asepsis and handwashing, antibiotic control and elimination of environmental sources. The overall reduction of P. aeruginosa colonized patients went from 23.9% to 15.4 (p=0.04 Fisher test). In conclusion, multiple drug-resistant bacteria continue to represent a major challenge in hematology units and efficacy measures which can contribute to the control of the outbreaks are: patient and staff cohorting, re-emphasis on strict hand-washing practices, and reduction of empirical antibiotic therapy.

CO076
EPIDEMIOLOGY, CLINICAL OUTCOMES AND ECONOMIC BURDEN OF NOSOCOMIAL CANDIDEMIA IN A GENERAL HOSPITAL

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For various reasons, the incidence of nosocomial candidemia (NC) has increased to an even greater extent than nosocomial bacteremia due to S. aureus and enterococci. It is estimated that Candida spp., which is currently the fourth most frequently isolated microorganism in nosocomial sepsis, cause more than 6,000 cases/year of NC in the USA, with an incidence of 2.43 cases per 100,000 inhabitants and a total treatment cost of more than 215 million dollars (Rentz et al., CID 1998). We therefore retrospectively evaluated the frequency of NC in the patients admitted to our hospital between 1.1.1994 and 31.12.1997. Of the 104 patients traced, 92 were evaluable (54 males and 38 females with a mean age of 61 ± 18 years). C. albicans was isolated in 66% of the patients and other Candida species in 34%. The majority were affected by AIDS (29%) or hematologic malignancies (15%), and 88% were carriers of a central venous catheter. Sixty-three percent of the patients were treated with fluconazole, 26% with amphotericin B and 2% withitraconazole; 11% of the cases were administered two drugs, and 20% did not receive any antifungal agent. Crude mortality was 43.5%, and attributed to Candida spp. infection in 82.5% of the patients. Mortality significantly correlated with the following parameters: the isolation of C. albicans, antibiotic polytherapy at the time of isolation (especially glycopeptides) and the non-use of antifungal therapy. Antimycotic consumption was evaluated for one year (1996) and represented the third item in the list of drug purchase costs: amphotericin B was the 67th most prescribed drug (DDD%) but third in terms of expenditure because of the high cost of the lipid-complexed amphotericin B formulations. Since September 2000 our hospital is currently carrying out a prospective 1-year study of the clinical and economic outcomes of all patients admitted to the ICU. Preliminary (6-month) results relating to NC concern seven patients (six with C. albicans and one with C. glabrata) aged 29-80 years. The length of stay in ICU of these patients (two of whom died) was significantly (p<0.05) longer than that of the controls not affected by NC, even after stratification on the basis of clinical severity (SAPS II); the daily workload (as measured by the Nine Equivalent Manpower Score [NEMS points]) of the subjects with NC was also significantly (p<0.05) different. ICU observed mortality was 13.8% in the controls and 28.6% in NC. During the six months of observation, the exclusively purchase cost of the antifungal drugs used in the ICU for antimycotic prophylaxis and therapy (patients with NC or other mycoses) was 43,554,000 italian lire (for a total of 444 DDDs), 61% for fluconazole, 38% for lipid-complexed amphotericin B formulations, and 1% for amphotericin B deoxycholate.

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CO077
CYTOMEGALOVIRUS INFECTION AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION. INCIDENCE, RISK FACTORS AND OUTCOME

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Background. Few data have been reported about incidence and morbidity from CMV in autologous bone marrow transplantation (ABMT), even if mortality for CMV disease has been reported. Unlike allogeneic bone marrow transplantation, the role of surveillance for CMV antigenemia and pre-emptive therapy is not established. Aim of the study. To evaluate incidence, risk factors and outcome of CMV infection in ABMT. Patients. CMV serum antigenemia was performed weekly in 97 ABMT, in 117 patients. All patients received transfusions of filtered and irradiated blood products and prophylaxis with acyclovir. Results. CMV reactivation was detected in 13 out of 117 autografts (11.1%). Five of them had non-Hodgkin’s lymphoma and received conditioning with BEAM therapy; six had multiple myeloma and received melphalan 140 or 200 mg/m2, two had acute myelogenous leukemia and were treated with busulphan or BUCY. All patients received peripheral blood stem cells; an average of 5.88×106/kg CD34+ cells were infused. They all were seropositive for CMV. They all received peripheral blood stem cells transplantation. Three pts received a CD34 selected graft. Antigen positive cells ranged from 1 to 30 cells/200,000 (average 9.1). The first postive antigenemia presented 17.3 days (range 0-68) after ABMT. Nine patients had fever; two of them with a Gram positive bacteria (Staphylococcus epidermidis and Haemolyticus). Two patients had pneumonia; none of them had radiological or clinical features suggestive of CMV pneumonia (one was a probable fungal infection). All patients were treated with ganciclovir (in 1 patient foscanet was associated). No patient suffered renal failure. No patient died of CMV infection. Ten patients are still alive and well. Three patients affected by myeloma died of disease progression 3, 15 and 25 months after ABMT. Conclusions. The incidence of CMV infection after ABMT is 11.1 %. Early preemptive therapy is effective in preventing morbidity and mortality from CMV disease.
CO078
BK VIRUS-ASSOCIATED CYSTITIS IN NON-MYELOABLATIVE AND CONVENTIONAL TRANSPLANT RECIPIENTS WITH SIMULTANEOUS CYTOMEGALOVIRUS REACTIVATION SUCCESSFULLY TREATED WITH CYDOFOVIR

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Hemorrhagic cystitis is a common complication following high-dose chemotherapy and bone marrow transplantation. Besides hydration and forced diuresis, there is no widely accepted treatment. Cydofovir is a nucleoside analogue, one of the most active compounds tested against polyomaviruses such as BK and JC virus, respectively. Two patients, a 35-year-old male with ALL and a 47-year-old male with AML, received an allograft from an HLA identical sibling using a myeloablative conditioning regimen. Both patients received cyclosporine and a short course of metotrexate for aGVHD prophylaxis. The ALL patient on day +4 became CMV antigenemia positive and because of neutropenia, was started on foscamet. On day +5 he developed severe macrohematuria with severe dysuria and voiding frequency of 14 times a day. BK virus (BKV) was isolated by means of PCR in the urine and the patient was started on Cydofovir at a dose of 5 mg/kg as a single infusion over 1 hour; simultaneously, probenecid and hydration were given p.o. and i.v., respectively as recommended by the manufacturer. The subsequent doses of cydofovir were not administered, because of sudden increase in serum creatinine (5mg/dL) which declined shortly thereafter with hydration. The symptoms declined progressively and by day +32 BKV and CMV antigenemia became persistently negative. The AML patient at day +30 complained of mild dysuria and BKV was detected by PCR in the urine. Because of the mild symptoms he received only hydration; dysuria resolved and by day +40 BKV become negative. One patient, 51-year-old male with metastatic melanoma underwent a non-myeloablative peripheral blood stem cell (PBSC) transplant from an HLA identical sibling, conditioned with fludarabine and melphalan and with postgrafting immunosuppression with cyclosporin and micophenolate mofetil. At day +102 the patient experienced severe dysuria, macrohematuria and blood clots in the urine. Ultrasound examination showed urinary retention as well as thickening of the bladder wall. BKV was detected in the urine by means of PCR as well as CMV, but the patient died soon thereafter before any treatment could be dispensed because of progressive disease. One patient, a 61-year-old female with relapsed multiple myeloma after 2 tandem autologous transplants, received a non-myeloablative allogenic PBSC transplant from an HLA identical sibling; conditioned with fludarabine and TBI, with cyclosporine and MMF as postgrafting immunosuppresion. At day +26 she complained of mild dysuria and BKV was detected by PCR in the urine. She only received hydration and was clinically monitored with resolution of symptoms and BKV negativization by day +40 by PCR. In conclusion, BK cystitis appears to behave either as a self-limiting cystitis with mild symptoms or as hemorrhagic cystitis requiring prompt treatment. This behavior can be observed also in the setting of non-myeloablative transplants. Two patients experienced a simultaneous reactivation of CMV both in the setting of conventional and of non-myeloablative transplants, showing that a reduced intensity of conditioning regimen does not allow a decline in infection surveillance. Cydofovir was successfully used to treat BKV hemorrhagic cystitis and simultaneous reactivation of CMV.

CO079
WEBGMS: EXPERIMENTAL WEB GLOBAL MEDICAL SYSTEM INTEGRATING A SATELLITE NETWORK IN THE INTERNET STRUCTURE FOR GIMEMA CENTERS IN ITALY

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A telecommunications network and proper software can make patient data available in remote sites enhancing co-operation between physicians in different institutions. The main objective of this project is the availability of a common base of multimedia data relevant to any patient in any institution connected to the network. Furthermore a streaming video server will broadcast informative video clips from companies (pharmaceutical and others interested in the field) evaluating the system for commercial use. In fact WebGMS aims at immediately promoting on the market interactive telecommunication applications based on existing platforms, delivering commercially viable services, such as information about new molecules and medicines, collaborations sessions, etc. Virtual community will be represented in the pilot phase by 35 Centers in Europe cooperating in the field of hematology and stem cell transplants. The underlying idea is that the information collected during the patient contacts with health-care structures is of paramount importance for decisions concerning the future treatment of the patient's diseases. Medical and paramedical personnel store and retrieve all the relevant data coming from any relevant event. Patient privacy is a primary issue, and is enforced through strict security checks. The multimedia patient record, the medical record, is the heart of the project. Medical data are stored on a central database (dB), hosted on a server at Telespazio S.p.A. in Rome. The application is based on the internet computing model using relational databases and runs within an ordinary web browser using the Java virtual machine, significantly reducing the client PCs configuration. The WEBGMS Project implements satellite telecommunication techniques such as multicasting IP, IP/DVB, etc. related to multimedia product distribution, co-operative work, marketing and product support. The proposed system has relevant economic advantages including: a) physicians treating patients in remote sites will share with Centers of Excellence all the data relevant for medical decisions and diagnosis; b) the medical protocols and guidelines for disease treatments can be shared. Remote site medical personnel can join Center of Excellence programs and associations. c) the collaborative work will be more than a mere tele-conference because the medical data will be validated and stored centrally and all information will be permanently available for patients and institutions; off-line consultation will also be possible; d) cryptography and the possibility to retrieve anonymous data will ensure patient privacy according to European Union privacy regulations and laws. In conclusion, technologies evaluated in this pilot project will allow
planning of cooperative activities such as clinical trials collecting and sharing data not from the classic CRF but directly from the original patient records.

This project has been co-funded by the European Space Agency in the framework of the ARTES 3 program (www.webgms.org).

CO080
MEDIMEDIA EUROPEAN COMUNITY PROJECT: ON-LINE HEMATOLOGY IMAGE ARCHIVE

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In the 4th Framework program, R&D Telematics Sector of the European Community has been funded the MEDIMEDIA project (Medical Images Integration for Multimedia European Databases Interconnection and Common Access project number HC 4013), based on integration of large databases of medical images concerning X-ray, endoscopy, CT scanner and hematology. Final results of this project, ended in June 2000, are available on line via internet (HTTP://MEDIMEDIA.GME.DE/FRAME.HTML). In October 2000 the Annual Technical Review (ATR 00) of the European Commission at point F (Exploitation) has evaluated results in this way: The project results have been proved to have a high educational level. The potential has been extensively addressed and could become a very useful tool for primary care physicians and medical students. Medical associations and universities should be encouraged to provide access to their databases and maintain the content according to the state of the art. In this project our department has participated creating the on-line hematology database. This archive includes digitized images from peripheral blood, bone marrow and other biological fluids as well as digitized reports from hematological analyzers. There are three connected parts: description of pathologies related to the images, diagnostic cell images from stained films and reports from the most advanced hematology analyzers, including numerical data, analogic flags and images of cell distribution patterns (scattergrams and histograms). Each image has a text explanation containing query words. In this way correlation with other MEDIMEDIA databases is possible using a common code system allowing a reciprocal enhancement through circular dialogue between other medical specialties. In detail the MEDIMEDIA hematology database is organized in four different typologies: (i) Clinical cases at diagnosis as well as in the follow-up; (ii) Blood diseases, anemias, leukemias, lymphomas, myeloproliferative myelodysplastic and lymphoproliferative diseases; (iii) Non hematological diseases, infectious, metastasis and bronchoalveolar fluids; (iv) Teaching issues, normal and pathological cases.

CO081
HEMATOLOGICAL HOME CARE: TEN YEARS’ ACTIVITY

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Hematology Department Azienda Ospedaliera Careggi and University of Florence

In September 1991 an experimental phase of Hematological Home Care (HHC) was started which was organized as Institutional Activity supported by Associazione Italiana Leucemie (AIL) starting from June 1992. The purposes of this activity were the reduction of admissions for patients without any chance of cure and the improvement of quality of life for patients with refractory/resistant disease or ending in death patients. During the time the assistant staff have been 8 hematologists and 6 nurses. Presently the staff is constituted by 3 hematologists (one is the person in charge of the activity), 2 nurses, volunteers and a psychologist. From September 1991 to March 2001, 184 patients entered the HHC service. The characteristics of these patients were: 101 were male (55%), 83 female (45%); the median age was 65 years (range 16-95). The hematological disease was a myelodysplastic syndrome or acute leukemia in 78 patients (42%), 38 patients (20%) were affected by a lymphoproliferative disease (lymphoma, Hodgkin’s disease, chronic lymphocytic leukemia), 31 patients (17%) presented the diagnosis of multiple myeloma, 22 patients (12%) were affected by myeloproliferative disease, 12 patients (7%) presented an acute lymphoblastic leukemia, 2 patients had the diagnosis of aplastic anemia and 1 was a hemophiliac patient. According to the condition at the moment of their inclusion in the HHC service: 122 patients presented a refractory or resistant disease, 35 were end-stage patients and 27 were patients discharged from hospital who needed to continue home supportive therapy such as transfusions or antibiotics. These patients usually have to be readmitted to the hospital in the future for further treatments. The median assistance period for each patient was 206 days (range 3 to 1985 days). The number of medical examinations was 5260 (median 29 for each patients) and the hours of nurse assistance were 10080 (median 55 for each patients). During the HHC assistance 112 cycles of i. v. monochemotherapy were administered, 46 of polychemotherapy and 27 bone marrow exams were performed. The supportive therapy was characterized by 1640 transfusions of RBC, 230 platelets aphereses and 98 cycles of intravenous antibiotics. All patients during the period of HHC have presented a complication which could have induced admission to hospital; 85% of these events were resolved at home. One hundred and sixty patients died and 127 (79%) died at home, 33 patients died during hospitalization. These data confirm the utility of HHC in the control of the costs of the National Health Service in Italy, moreover this system could permit patients to spend their last days close to parents and relatives.
hemostasis and thrombosis

CO082
GENETIC RISK FACTORS FOR ISCHEMIC STROKE IN THE YOUNG: INCREASED RISK AMONG THE CARRIERS OF THE G20210A POLYMORPHISM IN THE PROTHROMBIN GENE

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The role of the thrombophilic gene polymorphisms as risk factors for ischemic stroke is debated. Our study was aimed to evaluate the risk associated with two common thrombophilic polymorphisms (factor V Leiden and the G20210A polymorphism in the prothrombin gene) and the risk associated with the C677T polymorphism in the methylene tetrahydrofolate reductase (MTHFR) gene, possible cause of mild hyperhomocysteinemia. We investigated 239 patients (M/F 103/136) with a history of ischemic stroke before 50 years documented by CT or NMR scan; the mean age at the thrombotic event was 35 years (median 37, range 1 to 50). In 125 of them the ischemic event occurred in the absence of acquired risk factors (smoke, hypertension, diabetes, dyslipidemia, oral contraceptive intake, antiphospholipid antibodies). A control group of 494 healthy individuals (M/F 259/235, mean age 45 years, median 44, range 7 to 93) was also investigated. All individuals were genotyped for the presence of factor V Leiden (FV-L), the G20210A polymorphism in the prothrombin gene (FII-A), and the C677T polymorphism in the MTHFR gene. Among the patients we diagnosed 9 heterozygotes for FV-L (3.8%), 20 heterozygotes for FII-A (8.4%), 2 double carriers of FV-L and FII-A (0.8%), 2 homozygotes for FII-A (0.8%), and 48 homozygotes for the TT MTHFR polymorphism (20.1%). In the control group 11 were heterozygous for FV-L (2.2%), 1 homozygous for FV-L (0.2%), 13 heterozygous for FII-A (2.6%), and 80 homozygous for the TT MTHFR polymorphism (16.2%). The patients with no acquired risk factors and those with acquired risk factors showed similar prevalences of the FV-L carrier (5.6% vs. 5.3%, p = 0.10), FII-A carrier (8% vs. 12.3%, p = 0.28), and the TT MTHFR genotype carrier (20.8% vs. 19.2%, p = 0.87). No increase in risk for ischemic stroke was found in comparison with the controls among carriers of FV-L (odds ratio, OR, 1.9, 95% CI 0.8-4.4) or homozygotes for the TT MTHFR genotype (OR 1.3, 95% CI 0.9-1.9); the risk associated with the FII-A carrier was significantly increased (OR 4.1, 95% CI 2.1-8.3). Multiple regression analysis confirmed that FII-A carrier was the only genetic variable, among those investigated, significantly associated with ischemic stroke before 50 years of age (p < 0.0001).

CO083
INCREASED RISK OF PULMONARY EMBOLISM AMONG PATIENTS WITH DEEP VENOUS THROMBOSIS OF THE LEGS AND INHERITED ANTITHROMBIN III DEFICIENCY

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The risk of pulmonary embolism (PE) among patients with inherited thrombophilia is debated. In order to investigate the risk of embolization among patients with inherited thrombophilia suffering from deep venous thrombosis (DVT) of the legs we studied 522 unrelated patients with venous thromboembolic disease, 387 with isolated DVT of the legs (M/F 174/213, median age at the thrombotic event 35 years, range 1 to 84), and 135 with DVT and PE (M/F 77/58, median age 43 years, range 3 to 80). Diagnoses of DVT and PE were objectively proven; in 207 cases (40%) DVT occurred without a circumstantial risk factor. No patient had overt neoplasia or autoimmune disease. Among the patients with isolated DVT 133 (34.3%) had inherited thrombophilia: 2 antithrombin (AT) deficiency, 21 protein C (PC) or protein S (PS) deficiency, 71 factor V Leiden, 25 prothrombin G20210A, and 14 combined defects, in 3 cases associated with AT deficiency. Among the patients with PE as complication of DVT of the legs 47 (34.8%, p = 1.00) had inherited thrombophilia: 6 AT deficiency, 7 PC or PS deficiency, 19 factor V Leiden, 11 prothrombin G20210A, 4 combined defects. After stratification according to the different thrombophilic traits, the patients with AT deficiency resulted overrepresented in the group with PE (4.4%) in comparison with the group without PE (1.3%, p = 0.03), with a relative risk of embolization 3.4-fold increased (95% CI 1.1-10.9). After adjustment for other thrombophilic defects, the relative risk of embolization increased up to 8.2-fold (95% CI 1.7-39.8) in patients with DVT and isolated AT deficiency in comparison to those with DVT and normal genotype. No difference was found between the two patient groups as regards the distribution of other thrombophilic defects. The reason for the increased risk of embolization among the carriers of AT deficiency can be due to the more severe clinical penetrance of AT deficiency in comparison with other thrombophilic traits and to longer periods of inadequate anticoagulation during heparin treatment of acute thrombotic events in comparison with patients with normal AT levels. Thus, patients with DVT are recommended to be screened quickly for the presence of AT deficiency in order to have special care of the individuals at higher risk of embolization during the acute event.
MYH9 MUTATIONS IN PATIENTS AFFECTED BY MAY-HEGGLIN ANOMALY, SEBASTIAN SYNDROME, FECHTNER SYNDROME AND EPSSTEIN SYNDROME: GENOTYPE/PHENOTYPE CORRELATION


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May-Hegglin anomaly (MHA-OMIM 155100) is an autosomal dominant inherited disorder characterized by macrothrombocytopenia and granulocyte inclusions called Döhle-like bodies which consist of an amorphous area of cytoplasm containing clusters of ribosomes oriented along parallel microfilaments. Ultrastructural studies recognized a variant of MHA, the Sebastian platelet syndrome (SB5-OMIM 605249), characterized by Döhle-like bodies composed of highly dispersed filaments and few ribosomes. Macrophagocytosis and Döhle-like bodies are present also in Fechtner syndrome (FTNS-OMIM 153640) which is characterized also by nephropathy progressing to end-stage renal failure, sensorineural hearing loss and cataract. Until a few months ago, MHA-SBS and FTNS were considered as separate illnesses. The localization of MHA and FTNS loci to the long arm of chromosome 22 by our groups, allowed us to identify mutations of the non-muscle myosin IIA gene (MYH9) in both MHA-SBS and FTNS. Moreover, a mutation in the MYH9 gene has been found in a non-syndromic form of hereditary deafness (DFNA17) and we have recently demonstrated its involvement also in Epstein syndrome (EPS-OMIM 153650), an inherited disease differing from FTNS only by the absence of Döhle-like inclusions in granulocytes. In order to understand the pathogenesis of MYH9-related disorders, we have collected and analyzed a set of sporadic and familial patients (5 MHA-SBS, 5 FTNS and 3 EPTS) to establish a genotype-phenotype correlation. By direct sequencing, we have identified MYH9 mutations in 11 out of the 15 patients collected. Our results, as well as data obtained from recent publications of other groups, show that six mutations seem to be recurrent (R702H, R702C, T1155I, D1424H, E1841K, R1933X). In particular, the E1841K and R1933X substitutions have been reported in 8 and 7 MHA-SBS patients, respectively. In contrast, mutations at codon 702 seem to be specific to FTNS or EPTS cases: the R702H mutation has been identified in 2 EPTS patients and the R702C substitution in 2 FTNS cases. Interestingly, while the mutated thiol reactive group of R702C may lead to intermolecular disulfide bridges with a consequent formation of the inclusions typical of FTNS, the R702H does not allow the mutated proteins to aggregate and generate Döhle-like bodies, which are absent in EPTS. So far, no correlations between mutations affecting codons 1424 and 1155 and the different phenotypes have been reported. In fact, the missense mutation at codon 1424 (D1424H) previously identified in a FTNS family, has been found also in a MHA patient and, in association with an additional mutation (K910Q), in a sporadic case of EPTS. Finally the T1155I mutation, previously described in a MHA patient, has been recently identified in a FTNS family. In conclusion, the development of cellular and animal models as well as the genotype-phenotype correlation in a large number of patients will be important to add clues to the understanding of the non-muscle myosin IIA function and for a proper clinical management of patients with MYH9 mutations.

DÖHLE-LIKE LEUKOCYTE INCLUSIONS IN MYH9-RELATED DISORDERS ARE THE EXPRESSION OF AN ALTERED CYTOPLASMIC LOCALIZATION OF NON-MUSCLE MYOSIN IIA

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May-Hegglin anomaly (MHA) is a hereditary autosomal-dominant disorder characterized by macrothrombocytopenia and characteristic Döhle-like leucocyte inclusions. Fechtner syndrome (FTNS) is defined by the additional clinical findings of nephropathy, cataracts and sensorineural deafness. In both diseases we have recently identified mutations in the MYH9 gene, encoding the non-muscle myosin heavy chain IIA polypeptide, which is constitutively expressed in leukocytes and platelets. Nevertheless, the pathogenesis of the MYH9-related disorders still remains unknown. The aim of this study is to evaluate NM-MHCII localization in patients’ leukocytes and platelets and its possible correlation with the formation of Döhle-like inclusions. Patients and methods. The study included 8 patients (6 MHA, 2 FTNS) from 5 unrelated families (4 MHA, 1 FTNS), each one having a different MYH9 missense or nonsense mutation. Döhle-like inclusions were seen via morphological examination in 28-76% of neutrophils. Control subjects included: 7 healthy unrelated donors, 2 unaffected members from 2 of the MHA families, 4 affected members from a family with a hereditary macrothrombocytopenia-nephropathy-deafness syndrome (Epstein syndrome) and normal MYH9 sequence. The NM-MHCII localization was assessed by immunocytochemistry on peripheral blood smears, using the specific monoclonal antibody NGM2-MA. In the 2 FTNS patients ultrastructural studies with immunogold were performed. Results. Immunocytochemical analysis revealed a non-homogeneous distribution of NM-MHCII in the cytoplasm of patients’ neutrophils and platelets. The pattern of reactivity to the NGM2-MA was characterized by strongly positive cytoplasmic spots appearing in a negative or weakly stained background. A diffuse and homogeneous distribution of NM-MHCII was observed in leukocytes and platelets from all the control subjects. The altered NM-MHCII localization was seen in all neutrophils from all the affected individuals and was also observed in eosinophils, basophils and occasionally monocytes, while lymphocytes showed a diffuse cytoplasmic staining. The platelets were characterized by little spots usually localized at the cell periphery. In each patient the size, shape and localization of the cytoplasmic spots revealed in granulocytes by immunocytochemistry were comparable to those of the Döhle-like inclusions identified by morphological examination. Ultrastructural studies showed...
that microfilaments within the inclusion bodies were the subcellular structures recognized by the antibody. Conclusions. The expression of an abnormal NM-MHCIIA due to MYH9 mutation causes an alteration of its distribution in the cytoplasm of granulocytes and platelets. The formation of Dohle-like inclusions is related to the presence of zones of NM-MHCIIA accumulation in the cytoplasm. We previously pointed out that all identified MYH9 mutations are expected to have a role in the correct assembly or stability of the quaternary myosin complex: this could explain the altered NM-MHCIIA localization in MHA and FTNS patients and its possible aggregation into abnormal paracrystalline arrays. Until now the diagnosis of MHA and FTNS has been based on the morphological recognition of Dohle-like inclusions, which often proves difficult. Our results show that immunocytochemical analysis is a sensitive, specific and time-saving tool for the diagnosis of MYH9-related disorders.

CO086
FLOW CYTOMETRY INVESTIGATION OF RETICULATED PLATELET PERCENTAGE AND PLATELET ASSOCIATED IMMUNOGLOBULINS IN THROMBOCYTOPENIC PATIENTS: DIAGNOSTIC AND CLINICAL CORRELATIONS

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In a prospective study we have compared the usefulness of flow cytometry investigation of platelet associated immunoglobulins (PAIg) and reticulated platelet percentage (RP%) in the routine work-up of 145 thrombocytopenic patients. Reticulated platelets, which represent newly synthesized platelets, were assessed by flow cytometry using fluorochrome thiazole-orange. Seventy patients were classified as having an immunologic thrombocytopenia (IMT) while 31 and 44 patients had a diagnosis of infective (INT) and malignant (MAT) thrombocytopenia respectively. IMT was characterized by a lower PLT count (p=0.0386) and a higher RP% (p=0.0007). Platelet count significantly correlated with RP% among patients with less than 50 ×10^9/L platelets (PLT<50; p=0.005) and in the group of patients with PLT<50 and diagnosis of IMT (p=0.001). PAIg determination, in our hands, was a specific (88%) but not sensitive test (sensitivity 25.7%) with an overall test efficiency of 57.9%. The efficiency of PAIg determination in the PLT<50 group was 51.9%. RP% (cut off value of 4%) was a more efficient test (efficiency 69.6 %, p<0.0001) with a specificity of 51.4% and a specificity of 86.6%. At bone marrow examination (n=81), megakaryocytic hyperplasia and dysplastic features were associated with IMT (p=0.0002) and MAT respectively (p=0.0009). Response (complete and transient) to corticosteroid first line treatment in the PLT<50 group (n=35) was associated with higher RP% (p=0.003) and megakaryocytic hyperplasia (0.002) but not with PAIg positivity (p=0.063). Overall our analysis showed that PAIg investigation is an unnecessary and perhaps inappropriate test to be performed in the diagnostic investigation of thrombocytopenic patients. By contrast, RP%, which is a useful even though not perfect indicator of marrow megakaryocytopenesis, is a more efficient test which could be used not only for the diagnostic evaluation of thrombocytopenic disorders but also to predict the response (complete or transient) to first line treatment.

CO087
MOLECULAR GENOTYPING IN A COHORT OF SEVERE HEMOPHILIA PATIENTS WITH INHIBITORS

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The development of inhibitors against transfused factor VIII still remains the main complication of replacement therapy in patients with hemophilia A. This problem has been correlated in the past with the type of replacement therapy, the amount of therapy as well as to MHC status. To date none of these factors seems to fully explain the inhibitor formation. More recently patients carrying diverse FVIII gene defects have shown different proportions of inhibitor development. Large gene deletions, factor FVIII gene inversion and nonsense mutations display an incidence of antibodies formation of approximately 35%, compared with only 5-7% of patients with small gene deletions or missense mutations. The objective of this study was the identification of the molecular defects in a cohort of 21 severe A hemophiliacs with a history of inhibitor. FVIII gene inversion detection by multiplex long range PCR according to Liu (1998) revealed the presence of this common mutation in 4 (19%) patients. The other patients were analyzed by conformation sensitive gel electrophoresis (CSGE), a heteroduplex based method for nucleotide mismatch detection requiring amplification of the gene coding and regulatory sequences (26 exons and 5’ and 3’ flanking regions) as separate fragments. In 2 patients we were unable to obtain any PCR product for a portion of the FVIII gene (exons 2 to 25 and exons 5 to 10), suggesting a large deletion for both. By long range PCR, a specific product was obtained, using primers for 5’ and 3’ sequence flanking the breakpoint intronic regions. In the remaining patients 9 single nucleotide substitutions, 2 small deletions (4-pb and 7-pb), 2 insertions (1-pb) and 1 single nucleotide substitution in a splice junction were identified. The FVIII gene inversion represents a well known risk factor for inhibitor development but in this cohort it is not present as expected because of the bias in the selection criteria. Nevertheless other genetic defects probably interfering with the synthesis of a normal FVIII protein are well represented in this group especially deletions, insertions and nonsense mutations (57%). These results support the hypothesis that gene defects producing a severe phenotype can be frequently found in association with higher risk of inhibitor development.

CO088
MULTIPLE METHODS FOR THE CHARACTERIZATION OF HEMOPHILIA A AND B GENE MUTATIONS

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Hemophilia A (HA) and B (HB) are X-linked bleeding disorders that result from reduced or absent functional proteins in the plasma. Both display heterogeneity of mutations throughout the
hepatitis B virus infection does not play a major role in
hcv-related chronic hepatitis in patients with hemophilia

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Recent reports describe the possibility of occult hepatitis B in patients with chronic hepatitis C infection, with or without the presence of antibodies to HBV (anti-HBs and anti-HBe) and negative for hepatitis B surface antigen (HBsAg). This co-infection could play a role in the severity of the HCV related disease as well as in the response to interferon therapy (Cacciola et al. N Engl J Med 1999; 341:22-6). Nearly 100% of adult patients with hemophilia who were treated with non-virally inactivated plasma derived products before 1985 are chronically infected with HCV and a large proportion have been in touch with HBV and have antibodies to HBV generally considered markers of recovery from HBV infection. To explore occult hepatitis B in our cohort we have selected 77 patients followed up long-term at our Hemophilia Center. This cohort was chosen on the basis of well known transfusion history and because they were treated mainly with commercial products manufactured in USA and/or with crew cryoprecipitate produced locally in our blood bank. All patients were chronically infected by HCV, and screened for HIV and HBV markers. 53 (68%) had anti-HBs and/or anti-HBe; 4 (5%) were chronically infected by HBV (HBsAg positive) while 20 (27%) had no markers for HBV. Nine (12%) patients were HIV positive. Nested PCRs (first stage PCR products were used as template), using primers from the most conservative surface and core regions of the HBV, were used to test all these samples. Only one patient was found positive for both the regions (he was already known as being positive for HBsAg). Two patients were found positive for only one region (1 for the core and 1 for the surface region, but both were also chronic carriers of HBsAg). In conclusion we think that in patients with hemophilia the presence of occult HBV infection is a rare event, does not seem to be influenced by the type of replacement regimen used and does not represent a major risk factor for the severity of their HCV related disease and probably does not influence INF therapy. The possibility that the manufacturing of plasma derived products as well as the multiple and repeated administrations and infections can play a role in the definitive clearance of HBV in hemophiliacs should be taken into consideration.

CO089
HEPATITIS B VIRUS INFECTION DOES NOT PLAY A MAJOR ROLE IN HCV-RELATED CHRONIC HEPATITIS IN PATIENTS WITH HEMOPHILIA
remission (normal FVIII:C and no detectable inhibitor) was obtained in 16/19 patients, a partial remission (inhibitor level <10 BU/µL or decrease of 50% if the baseline inhibitor was <10 BU/µL) in 3 patients; 6 patients relapsed but were rescued. A stable complete remission was obtained in 18/19 patients. In two patients the inhibitor disappeared spontaneously after 2 and 4 months. In the patients with the 1st complete remission the time to response was not related to the inhibitor titre. The occurrence of relapse was not related to the inhibitor titre or the time to response. Comments. aPTT values preoperatively are frequently overlooked. The prolonged PTT, already present at the time of the procedure, should have alerted to the presence of the inhibitor. P is the initial treatment of choice. The complete remission was high (80%) and the relapsed patients were rescued. Chemotherapy is indicated in resistant or relapsing patients.

**Cytogenetics**

CO091
CHROMOSOMAL ABERRATIONS DETECTED BY COMPARATIVE GENOMIC HYBRIDIZATION TYPICAL B-CLL: CORRELATION WITH CD38 EXPRESSION

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B-cell chronic lymphocytic leukemia (B-CLL) can be classified as typical or atypical by means of morphologic or immunophenotypic criteria. It has been already demonstrated that trisomy 12 defines a group of B-CLL classified as atypical and which showed an aggressive clinical course. Patients with typical B-CLL show a heterogeneous clinical behavior. The aim of this study is to identify chromosomal aberrations able to discriminate between patients who will experience a good or poor survival or to identify an aberration which defines typical B-CLL. Forty-five B-CLL patients were examined for partial or total chromosomal gains/losses by comparative genomic hybridization (CGH). Malignant B cells, purified from peripheral mononuclear cells by removing CD3+ T cells, were analyzed to improve the sensitivity of CGH. Only CD19+, CD5+, CD23+, weak Ig- and high level Bcl-2-expression patients (typical phenotype) were enrolled in this study. All samples were also screened for CD38 expression. Thirty-eight percent of patients showed chromosomal gains or losses, a percentage lower than described in mixed typical and atypical B-CLL (48-64%). This technique was not able to detect in this group of typical B-CLL patients aberrations different from those described in mixed B-CLL. In fact, the most common aberration (16%) was partial of total gain of chromosome 12, previously reported as identifying atypical B-CLL. The second most common aberration (9%) was loss of 11q14-q23. Both these aberrations have been previously described to be correlated with poor prognosis. Loss of 13q14-q31 was detected in 6% of patients. This aberration has been associated with a good prognosis. Comparison between the CGH and CD38 expression data showed that 89% of patients without any detectable aberration display low (<30%) CD38 expression, as well as the three patients bearing 13q deletions. On the contrary, 82% of patients with aberrations already described to correlate with poor prognosis display high CD38 expression. One of the two patients with poor prognosis-related aberrations and low CD38 expression showed adverse clinical features and needed continuous intensive treatment. These data indicate that the combination of chromosomal aberrations detected by CGH and CD38 expression may be a useful tool to discriminate between good and poor prognosis patients.
CO092
20q- CHROMOSOME UNDERLYING A WHOLE ARM TRANSLOCATION BETWEEN CHROMOSOME 20 AND 21. FLUORESCENT IN SITU HYBRIDIZATION CHARACTERIZATION OF A NEW ANOMALY IN MDS/AML

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Genetic similarities between myelodysplasia (MDS) and acute myeloid leukemia (AML) emphasize a continuum between these two malignancies. We identified a new type of chromosomal rearrangement in one MDS-refractory anemia with excess of blasts (RAEB) and one AML, both at diagnosis. Patient 1: A 52-year-old woman was diagnosed as a de novo AML. M1 with massive (90%) bone marrow infiltration. Karyotype was 46,XX, +8, der(20), -21, +mar. Patient 2: A 73-year-old man was admitted because of bicytopenia (WBC=2.5×10^9/L; Plt=80×10^9/L). Diagnosis of AML was based on bone marrow morphology. Karyotype was 46,XY, (7;18)(q22;q11), -21, +mar. In the first case the der(20) was a whole arm translocation between the short arm of chromosome 20 and the long arm of chromosome 21. Comparative genomic hybridization (CGH) experiments confirmed the gain of a whole chromosome 8 and showed loss of DNA in the interstitial part of 20q. This unbalanced translocation was further investigated with centromeric and locus-specific probes (YACs 834H3, 819A9, 742F19) for 20q arm, showing loss at bands 20q12, 20q13.11, and 20q13.12. The centromeric probe for chromosome 20 was retained on der(20), while the PAC probe 81F12 for the subtelomeric region was translocated to the small marker chromosome 16. Case #1: female, 41 yrs, M4 eo, karyotype 47XX,del(5)(q12),11, der(16)del(16)(q22) t(11;16)(p11;p13), +mar 1, +mar 2. Case #2: male, 68 yrs, M2 with bone marrow eosinophilia and myeloid precursors with baso-eosinophilic granules, karyotype 46 XY, t(8;16)(q22;p13). Both patients, treated with intensive chemotherapy (Ida-FLAG, FLAG), have obtained complete remission. In the first case, paintings for chromosome 5, 11 and 16 (Oncor), showed a three-way complex translocation, t(5;11;16), resulting from a translocation of chromosome material deriving from 5q on chromosome 11, and material of chromosome 11 on chromosome 16. The use of cosmid zit 27/29 and zit 14/18 for the 31 and 51 portion of gene M.YH11 (16p13), respectively, has identified a pericentric inversion of chromosome 16, inv(16)(p13q22), masked in the complex translocation. In the second case, paintings for chromosome 8 and 16 confirmed the presence of material of chromosome 8 on der(16), while material of chromosome 16 was present on chromosome 21 which appeared normal at conventional banding. Such data were confirmed by painting of chromosome 21 (Oncor) which completely marked the normal chromosome 21, partially der(21) and the telomeric region of the long arm of der(8). Even in this second case, therefore, FISH by paintings showed a three-way complex translocation t(8;16;21)(q22;p13;q22). The use of specific probes for gene M.YH11 instead excluded a masked inv(16)(q22p13) whilst defining the point of attack of chromosome 8 on 16p in centromeric position regarding M.YH11. The FISH study with pac 1107L6 (kindly provided by Dr. M. Rocchi, Institute of Genetics, University of Bari), for ALM1 gene (21q22) and pac c1164,2 for ETO gene (21q22), showed a fusion sign on der(8) typical of the AML1/ETO rearrangement. Therefore, in both of these cases, the FISH study has made it possible to identify the presence of molecular rearrangements typical of AML FAB subtypes. It is interesting to note that the cytomorphological characteristics (M4 Eo) in the first patient implied an inv(16)(p13q22), whilst the immunophenotype of blast cells (CD13/CD33 plus CD19 positive) in the second patient is commonly associated with the translocation t(8;21)(q22q22).


CO094
TRISOMY 12 AND t(14;22) IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA


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Recent clonal cytogenetic abnormalities are well described in B cell chronic lymphocytic leukemia (B-CLL). Recent studies have clearly highlighted their prognostic significance in a large group of B-CLL patients suggesting that cytogenetic analysis should be performed at diagnosis in all the cases. We report here a case of B-CLL showing, at the cytogenetic level, trisomy 12 and translocation t(14;22)(q32; q11). Case Report. A 51-year-old

CO093
TYPICAL REARRANGEMENTS OF ACUTE MYELOID LEUKEMIA LOCALISED BY FLUORESCENT IN SITU HYBRIDIZATION IN COMPLEX KARYOTYPES

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Fluorescent in situ hybridization (FISH) experiments for the research of cryptic inversion of chromosome 16 were undertaken in 2 cases of acute myeloid leukemia (AML) characterized by bone marrow eosinophilia and complex karyotype involving chromosome 16. Case #1: female, 41 yrs, M4 eo, karyotype 47XX,del(5)(q12),11, der(16)del(16)(q22) t(11;16)(p11;p13), +mar 1, +mar 2. Case #2: male, 68 yrs, M2 with bone marrow eosinophilia and myeloid precursors with baso-eosinophilic granules, karyotype 46 XY, t(8;16)(q22;p13). Both patients, treated with intensive chemotherapy (Ida-FLAG, FLAG, FLAG), have obtained complete remission. In the first case, paintings for chromosome 5, 11 and 16 (Oncor), showed a three-way complex translocation, t(5;11;16), resulting from a translocation of chromosome material deriving from 5q on chromosome 11, and material of chromosome 11 on chromosome 16. The use of cosmid zit 27/29 and zit 14/18 for the 31 and 51 portion of gene MYH11 (16p13), respectively, has identified a pericentric inversion of chromosome 16, inv(16)(p13q22), masked in the complex translocation. In the second case, paintings for chromosome 8 and 16 confirmed the presence of material of chromosome 8 on der(16), while material of chromosome 16 was present on chromosome 21 which appeared normal at conventional banding. Such data were confirmed by painting of chromosome 21 (Oncor) which completely marked the normal chromosome 21, partially der(21) and the telomeric region of the long arm of der(8). Even in this second case, therefore, FISH by paintings showed a three-way complex translocation t(8;16;21)(q22;p13;q22). The use of specific probes for gene MYH11 instead excluded a masked inv(16)(q22p13) whilst defining the point of attack of chromosome 8 on 16p in centromeric position regarding MYH11. The FISH study with pac 1107L6 (kindly provided by Dr. M. Rocchi, Institute of Genetics, University of Bari), for ALM1 gene (21q22) and pac c1164,2 for ETO gene (21q22), showed a fusion sign on der(8) typical of the AML1/ETO rearrangement. Therefore, in both of these cases, the FISH study has made it possible to identify the presence of molecular rearrangements typical of AML FAB subtypes. It is interesting to note that the cytomorphological characteristics (M4 Eo) in the first patient implied an inv(16)(p13q22), whilst the immunophenotype of blast cells (CD13/CD33 plus CD19 positive) in the second patient is commonly associated with the translocation t(8;21)(q22q22).


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Department of Hematology “A. Sclavo” Hospital, University of Siena

Recent clonal cytogenetic abnormalities are well described in B cell chronic lymphocytic leukemia (B-CLL). Recent studies have clearly highlighted their prognostic significance in a large group of B-CLL patients suggesting that cytogenetic analysis should be performed at diagnosis in all the cases. We report here a case of B-CLL showing, at the cytogenetic level, trisomy 12 and translocation t(14;22)(q32; q11). Case Report. A 51-year-old
female was referred to the Division of Hematology of Siena on September 19th, 1996 because of a 3-year history of persistent lymphocytosis exceeding 5×10⁹/L. On physical examination, small lymphnodes were noted in cervical and axillary regions, without hepatosplenomegaly. Her hemoglobin concentration was 12.9 g/dL, the white blood cell and platelet count were 1.10×10⁹/L, with 75% of typical small lymphocytes and 185×10⁹/L, respectively. The lymphocytes showed the following phenotype: CD5+, CD19+, CD23+, sIg+ with low fluorescence intensity (MIF 16). Cytogenetic analysis of peripheral blood cells was: 47, XX, +12, t(14;22) (q32;q11) in 4/50 and 46, XX in 46/50 metaphases analyzed. The bone marrow aspiration showed 40% of lymphoid cells, mostly composed of typical small lymphocytes with clumped chromatin and absent nucleolus. The bone marrow biopsy showed a nodular pattern of infiltration. The course of disease has been indolent and the patient has received no therapy. Discussion. Trisomy 12 has been found as a recurring chromosomal abnormality in B-CLL in several studies, in most of them being associated with atypical morphology, shorter treatment-free intervals, and shorter overall survival. The translocation t (14;22) (q32;q11) that involves breakpoints within the immunoglobulin heavy chain locus on chromosome 14 and the lambda light chain locus on chromosome 22 has been reported only in another case in the 1981 by Nowell et al. The translocation involving chromosome band 14q32, which is frequently observed in other type of non-Hodgkin’s lymphomas, is a rare event in B-CLL, and in the early cytogenetic studies was associated with an inferior survival. Our patient and the other case previously reported, differently from what usually described, showed a very stable disease and the patients did not receive any therapy. This could be partially explained by the fact that in the past, 14q aberrations were frequently due to the translocations t(11:14) and t(14:18), today considered the hallmark of mantle cell and follicular lymphomas, respectively.


CO095
A NOVEL RECURRENT TRANSLLOCATION T(11;14)(P11;Q32) IN SPLENIC MARGINAL ZONE B-CELL LYMPHOMA

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A novel recurrent translocation t(11:14)(p11;q32) was found in 3 patients with splenic marginal zone B-cell lymphoma (MZBCL). Fluorescence in situ hybridization (FISH) studies with IgH probes revealed in all cases involvement of the IgH locus, with breakpoint downstream of the IGHV sequences. Partner genes at 11p11 were not identified. The translocation defined the stemline in 2 patients, who carried additional cytogenetic aberrations, including a 17p deletion, present in both cases. In one patient a 7q− chromosome was the primary cytogenetic defect, the t(11:14) being found in 4 out of 11 abnormal metaphase cells at the time of transformation into high-grade MZBCL. Hematological features in all cases included splenomegaly with peripheral blood (PB) involvement by a monoclonal B-cell population consisting of lymphocytes with villous projections and several blast-like cells. The immunophenotype was CD19+, CD22int+, CD23-, CD10-, CD5, surface IgG+. A bone biopsy in one patient revealed an interstitial infiltration with an in intrasinusoidal pattern of growth. Histological studies on spleen specimens in 2 patients showed an expanded marginal zone, with small lymphocytes and several blast-like cells. One patient had a therapy-demanding disease, with partial, short-lasting responses to cytotoxic treatment; one patient transformed into a high-grade MZBCL involving the gut, the PB and the bone marrow two years after diagnosis; one patient was unresponsive to cytotoxic treatment and underwent splenectomy. The t(11:14)(p11;q32) may define a subset of splenic MZBCL with a high-grade component and a relatively aggressive clinical behavior.

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The chromosomal rearrangement leading to inv(16) (p13q22) is closely associated with FAB M4EO AM L subtype and results in the transcription of a specific CBFβ/MyH11 fusion mRNA. Reverse transcription polymerase chain reaction (RT-PCR) for CBFβ/MyH11 transcript can be used for diagnostic purposes as well as for the detection of minimal residual disease (MRD). Qualitative RT-PCR studies of MRD have so far produced conflicting results with some patients in long term clinical remission being persistently positive; thus, qualitative RT-PCR seems of limited prognostic value in a consistent proportion of cases. We have evaluated retrospectively the MRD in a large series of CBFβ/MyH11 positive AM1s employing both qualitative and quantitative (REAL TIME RT-PCR) approaches. A total of 183 samples from 38 patients were examined with a median follow-up of 27.5 months; 15 pts. relapsed at various time during the follow-up. In qualitative studies, carried out by nested RT-PCR assay, sequential BM samples were positive in all patients in CR after induction and consolidation therapy; however, follow-up samples were found persistently negative (except one case) in patients remaining in continuous CR (CCR) for more than 12 months; in contrast, the detection of CBFβ/MyH11 transcripts after 1 year of CR was associated with subsequent relapse. Fifteen patients were sequentially evaluated by quantitative REAL-
The specific chromosomal translocation t(14;18) is associated with follicular lymphoma and the resulting fusion gene (Bcl2/IgH) can be tested at the DNA level. The PCR technique provides an excellent opportunity to monitor residual disease in specific situations, i.e., CR after radiotherapy, chemotherapy and after high-dose chemotherapy supported by bone marrow or PBSC transplantation. Quantitative results might help to gain more information on minimal residual disease by PCR analysis of bone marrow and/or circulating blood cells. Fifteen patients affected by relapsed or refractory follicular non-Hodgkin’s lymphoma (WHO grade I-2; M:F=4:11; median age 45 years, range 28-60) have been treated with autologous transplant of PBSC (n=13) or allo-BMT from HLA-identical sibling donors (n=2). The patients have been evaluated by PCR to detect the Bcl2/IgH rearrangement (10 M BR and 1 mcr type positives). The PCR positive patients have been also studied with competitive PCR before and after the transplant procedure. The competitive PCR has been performed with a multiple competitor carrying the sequences to amplify the Bcl2/IgH (M BR and mcr type) and the Bcl1/IgH rearrangement and the β-globin gene. Two primer pairs specific for each rearrangement were included in the competi-
tor because the size of translocation products may vary from patient to patient. If the translocation and the competitor products have similar size, the second primer set can be used to give different size products. The constructed competitor was used in a series of quantitative validation experiments to evaluate accuracy, reproducibility, analytical range and detections limits. Forty-seven cellular samples of the 11 patients harboring t(14;18) were tested with PCR before transplant and 23 samples (49%) resulted PCR positive. After transplant 15 samples (38%) out 39 proved to be PCR positive. Three patients treated with the positive selected CD 34+ autologous cells (PCR negative) have a PCR negative follow-up of +20, +25, and +42 months. One patient treated with selected CD 34+ cells PCR positive, was persistently PCR positive and then relapsed. Both patients treated with allo-BMT are PCR negative (+29, +44 months). Patients treated with unmanipulated PBSC had alternate positive/negative PCR results. The quantitative PCR study is ongoing and preliminary results show a significant reduction of the rearranged DNA in the post transplant and a trend to reduction over time. A longer follow-up will provide more precise information on the outcome of these patients.

C0099 EVALUATION OF MINIMAL RESIDUAL DISEASE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: LABORATORY STRATEGY TO IDENTIFY MOLECULAR PROBES FOR DIAGNOSIS, MONITORING AND RISK ASSESSMENT WITHIN A NEW PROSPECTIVE CLINICAL TRIAL

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Recent clinical trials performed in pediatric ALL provided striking evidence on the crucial role played by molecular evaluation of minimal residual disease to define risk groups, the clinical outcome and possibly, therapeutic options. With the aim to include MRD evaluation during the treatment of each patient, a new collaborative Phase II multicenter clinical trial for the treatment of adult ALL (LAL 09/2000) was started at different Italian hematologic institutions (Bergamo, Bolzano, Brescia, Milan, Monza, Noale, Vicenza, Venice) in March 2000. The challenge of this study is to follow each patient during the treatment course by an adequate molecular PCR analysis and this will be instrumental to an early identification of patients at high risk of leukemia relapse. The definition of risk is based upon the molecular results obtained on two consecutive samples collected during the treatment protocol: the MRD positivity in one or both samples identified the high risk patients and the MRD negativity in both samples identified the low risk patients. Patients bearing BCR-ABL gene rearrangement or MRD positive are treated by allogeneic transplantation whenever possible or if a donor is not available, by repeated cycles of high dose chemotherapy supported by autologous transplants with in vitro purged hematopoietic stem cells. Patients proven MRD negative are treated by maintenance chemotherapy. During the first eleven months since protocol activation, 38 patients have been enrolled to the study, and all of them were molecularly analyzed to identify one or if possible two probes for the molecular diagnosis and MRD evaluation. All patients were molecularly tested for the most common chromosomal rearrangements according to the European Concerted Action on MRD (JM van Dongen et al., Leukemia 1999; 13:1901-8) and amplified by PCR for the most frequent TCRγ, TCRδ and IgH rearrangements with the appropriate positive and negative controls. The monoclonal amplifications are sequenced and probes designed on the junctional patient-specific region. The probes are then tested for specificity and sensitivity. So far, 13 patients have been found BCR-ABL positive and one patient showed a MLL-AF4 gene rearrangement. Twenty-one patients had a clonal rearrangement of TCRγ, 13 of TCRδ, and 19 of IgH. In 25 patients (66%) two molecular probes were obtained and only for three patients did we fail to identify a suitable molecular probe. The mean sensitivity level of the TCR or IgH probes was $10^{-4}$ (range $10^{-3}$-$10^{-5}$) as determined by semiquantitative dot blot assay using 32P labeled oligonucleotides. At this moment 13 patients have been evaluated for risk category and 9 of them assigned to the high risk group. Moreover, all these 13 patients had the possibility to collect G-CSF mobilized, autologous peripheral blood stem cells which after in vitro purging were considered PCR negative in 10 cases. These preliminary results indicate the feasibility of this MRD based study in adult ALL and its wide applicability to a multicenter clinical trial.

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multiple myeloma

CO100
ANTI-IDIOTYPE IMMUNOTHERAPY INDUCES RECOVERY OF POLYCLONALITY OF THE TCRBV REPertoire IN MULTIPLE MYELOMA PATIENTS

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Specific active immunotherapy is the subject of clinical experimentation in multiple myeloma (MM) as a form of maintenance management during remission. Its effects, however, are still uncertain. We have therefore investigated the degree of T cell clone diversity in 8 patients following the administration of an idiotype vaccine during remission induced by high-dose chemotherapy and transplantation of autologous peripheral blood progenitor cells. Peripheral and marrow blood samples were collected before and up to 12 months after vaccination. Total RNA was extracted from mononuclear cells and converted into cDNA. Specific PCR reactions were then carried out to determine the quality of the targets. Next, two consecutive PCR reactions were performed with primers specific for the TCRBV families and a TCRBC consensus tract to provide amplified products corresponding to all the possible T cell clones with a sequence of interest. Computerized analysis with an automatic DNA sequencer and specific software after separation of the fragments on an acrylamide gel showed that those for each TCRBV family displayed four length distribution patterns: Gaussian (NOR), reactive (REA), deteriorated (DET) and single peak (SP). NOR and REA alone were present in the controls (healthy blood donors) and were therefore regarded as normal and indicative of the polyclonality of the TCRBV repertoire. DET and SP were considered abnormal and indicative of oligoclonality. Vaccination induced an increase in polyclonal TCRBV families from 74.7 ± 4.3% prior to vaccination to 88.2 ± 3.3% (p = 0.0036) 1-5 months after the last administration. This increase was considerably from one patient to another, with mean values ranging from 53% to +142% during the observation period. Assessment of the relation between this variability and the mean reduction of the TCRBV DET families over time showed that this reduction was inversely correlated with the increase in the NOR + REA families (r = -0.824; p=0.012), whereas the percentage of the TCRBV DET family reduction was inversely correlated with the duration of remission (r = -0.818; p=0.0137). These findings show that anti-idiotype vaccination modulates the TCRBV repertoire of MM patients by acting on a part which leads to recovery of the receptor diversity typical of normal subjects, and also that this recovery is correlated with the duration of remission. At present, however, recovery of the part of the repertoire most severely altered and primarily composed of monoclonal families is more difficult to achieve.

CO101
CLINICAL AND IMMUNOLOGICAL FEATURES OF THE LONG-TERM MONITORING OF MULTIPLE MYELOMA PATIENTS AFTER IDIOTYPE-SPECIFIC VACCINATION

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This paper describes the long-term clinical and immunological effects of an experimental idiotype-specific vaccination protocol administered to multiple myeloma (MM) patients in first remission after high-dose chemotherapy and infusion of peripheral blood stem cells. Fifteen patients were enrolled between August 1995 and July 1998. Two did not complete the treatment owing to early relapse. Of the 13 that did, 2 have maintained their remission for 34 and 55 months since the initial vaccination, whereas the remaining 11 relapsed over the course of 9 to 50 months. These clinical responses were compared in a case-match analysis to those of 13 patients in first remission treated with monthly high-dose steroid courses in addition to or as an alternative to three-weekly s.c. administration of IFN-α. Matching was performed with respect to age, disease stage at diagnosis and number of previous high-dose melphalan courses. The median progression-free survival, measured between the date of the first remission and that of the first treatment in relapse, was 37 months in the vaccinated patients and 24 months in the controls (p<0.05) over a mean follow-up of 83 and 52 months, respectively. The median survival is 81 months in the vaccinated patients and 38 months in the control group (p<0.05). Immunological monitoring showed that all patients produced type IgM and type IgG anti-KLH antibodies, whereas no anti-Id antibodies were found. Anti-GM-CSF antibodies were noted in 40%, but had no effect on white cell numbers and function. Increased serum IgE levels were observed in 70% of vaccinated patients and displayed an idiotype-specific reactivity in those with IgG MM. The cell response to KLH was positive in 82% of cases when assessed with in vitro T cell proliferation tests, and in 100% when delayed type hypersensitivity (DTH) skin tests were used. Idiotype-specific DTH were documented in 84% of the vaccinated patients, and DTH remained positive for more than one year after the last administration in 3 cases. It is thus clear that idiotype-specific vaccination can result in long-lasting tumor-specific and non-tumor-specific immune responses. These immunological results are also associated with promising clinical effects that could well be made the subject of randomized trials comparing their efficacy with that of conventional maintenance treatments.

CO102
NEW MECHANISMS OF FIBROBLAST GROWTH FACTOR RECEPTOR 3 DEREGULATION IN MULTIPLE MYELOMA PATIENTS

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The t(4;14)(p16.3;q32) chromosomal translocation can be detected by fluorescence in situ hybridization (FISH) in approximately 20% of multiple myeloma (MM) patients, and results in the ectopic expression of fibroblast growth factor receptor 3 (FGFR3) proto-oncogene from der4. MM cells subsequently select activating mutations in the translocated FGFR3, suggesting that its signaling pathway plays an important role in tumor development and progression. We set up a sensitive and straightforward real-time RT-PCR method to quantify FGFR3 messenger RNA in 78 MM patients at diagnosis. Ten of them (13%) showed FGFR3 overexpression. These patients were further examined to assess whether FGFR3 overexpression was associated with activating mutations. We amplified and sequenced five distinct cDNA fragments containing the codons usually affected by amino acid substitutions: namely, codon 248, the entire transmembrane domain (codons 371, 373, 375, and 380), codon 540, codon 650 and codon 807. In one patient - with t(4;14) as confirmed by fluorescence in situ hybridization (FISH) - we detected a single base-pair mutation in codon 248 in the form of a C-to-T transition (CGC → TGC) leading to an Arg-to-Cys amino acid substitution. This mutation results in strong ligand-independent activation of the receptor and, within the germline, causes the most severe form of dwarfism (i.e. thanatophoric dysplasia type I).

In another patient - without t(4;14) as assessed by FISH - primer-specific amplification of sequences encompassing codon 248 revealed, as well as the expected fragment (WT), corresponding to the FGFR3-IIIc isoform, three additional abnormal-sized fragments (AT-I, -II, and -III). Sequence analysis showed that the latter should correspond to truncated transcripts originating from cryptic splice donor sites located within exon 7, at positions 829 (AT-I), 868 (AT-II), and 892 (AT-III). As a consequence, these shorter transcripts had deletions of 39, 63 and 102 nucleotides, respectively. Thus, the translation products of AT-I, -II and -III can be predicted as truncated proteins lacking 13, 21 and 34 amino acids in the third immunoglobulin-like domain. Analysis of the genomic DNA sequences surrounding the cryptic splice donor sites did not show any mutation or deletion. However, these sequences were identical in 7 (CAAG/GTG), 4 (CAAG) and 11 bases (CAAG/GTGGCCC), respectively, to the normal exon 7/intron 7 boundary (...CAAG/GTGGCCC...). This suggests that these three ATs all arose from the activation of cryptic splicing sites and that such errors of exon definition occurred mechanistically in trans. Our results show two novel mechanisms of FGFR3 deregulation in MM patients. Moreover, they seem to indicate that mutations in the overexpressed FGFR3 could occur at an early stage of tumorigenesis and even independently from the presence of the translocation. For both these reasons, the frequency of FGFR3 deregulation in MM patients with or without the t(4;14) translocation could be underestimated.

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**COI013**

**EVALUATION OF THE ABILITY OF MONOCYTE-DERIVED DENDRITIC CELLS TO ENDOCYTOSE TUMOR-DERIVED NECROTIC MATERIAL IN MULTIPLE MYELOMA**

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Specific active immunotherapy studies have shown that tumorspecific immune responses can be generated in multiple myeloma, but are not powerful enough to reduce the tumor mass. Dendritic cells (DC) are of crucial importance in the enrollment and activation of cytolytic T lymphocytes (CTL) through their exclusive cross-priming effect, which requires endocytosis of tumor-derived material and the subsequent maturation of DC themselves induced by interaction with the CD40 ligand (CD40L), a molecule expressed by activated CD4+ lymphocytes. This paper examines in MM the ability of peripheral monocyte-derived DC (Mo-DC) to endocytose necrotic material derived from myelomatous cells by repeated freeze-thawing courses. The cytoplasmic localization of the endocytosed material was confirmed by flow cytometry and confocal microscopy, using supravital stains for the Mo-DC, membrane staining for the HLA-DR they expressed and intracytoplasmic staining for the kappa or lambda light chains expressed by the tumor cells. The percentage of endocytosing Mo-DC was similar in MM and the normal controls (51.6% vs 39.5%, p<0.28) when myelomatous lines were used, but significantly lower in MM when both fresh autologous myelomatous cells (9.2% vs 19.3%, p<0.039) and fresh allogeneic myelomatous cells (12.72% vs 19.3%, p<0.058) were employed. Endocytotic activity varied in MM since patients generated both functionally normal and functionally defective Mo-DC. It was also found that myelomatous cells were not equally susceptible to endocytosis. In some cases, substantial quantities of necrotic material were endocytosed by the Mo-DC of normal subjects and less by those of MM patients, whereas in others the material was virtually resistant to endocytosis by both sets of Mo-DC. These findings show that the endocytosis of tumor-derived necrotic material is generally defective in the Mo-DC of MM and that individual variations exist in both level of Mo-DC functional efficiency and the susceptibility to endocytosis of myelomatous cells. These functional features may be partly responsible for determining a patient’s level of immune competence. They must thus be considered in the formulation of any kind of active immunotherapy.

**COI014**

**MULTIPLE MYELOMA: THALIDOMIDE EFFECTS ON APOPTOSIS**

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Recent evidence suggests that thalidomide (Thal) can induce marked and durable response in multiple myeloma (MM) patients based on several partially known mechanisms of action of this drug, which includes induction of apoptosis. Therefore we decided to investigate: a) the in vitro role of Thal on induction of
Cytokine (TRANCE) in Multiple Myeloma

Role of Tumor Necrosis Factor-Related Activation-Induced Cytokine (TRANCE) in Multiple Myeloma

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The interaction between myeloma cells and bone environment has a pivotal role in the pathogenesis of bone lesions and plasmacytoma growth. Two factors, produced by stromal cells, the tumor necrosis factor-related activation-induced cytokine (TRANCE) and its antagonist osteoprotegerin (OPG), have been recently identified as critical in the regulation of osteoclast activity and apoptosis. In this study we investigated the role of this system in multiple myeloma (MM). First, we found that neither several human myeloma cells lines (HMCL) nor fresh myeloma cells purified from 26 MM patients did express TRANCE directly. In a co-culture system, we demonstrated that HMCL upregulate TRANCE and downregulate OPG in primary human bone marrow stromal cells (BSC), through the cell-to-cell contact, with the involvement of very late antigen-4 (VLA-4) but not of interleukin-6 (IL-6). Human CD34+ derived osteoclastogenesis was stimulated by HMCL only in the presence of BSCM and reduced by rhOPG. In addition, BSC isolated from MM patients overexpressed TRANCE mRNA compared to normal subjects. Using an immunohistochemical staining, a reduction of OPG expression in trabecular osteoblasts, with an increase of TRANCE in stromal cells was observed in MM patients with bone lesions compared to patients without osteolytic or to controls. (OPG+ cell%±SE: 3.2±0.7 vs. 6.5±1.6 and 6.7±1.2 respectively, p<0.05; TRANCE+/mm²±SE: 2.1±0.03 vs. 0.91±0.08 and 0.93±0.18 respectively, p<0.05).

OPG serum levels were reduced in MM patients compared to normal subjects (mean±SD: 26.6±4.8 vs.18.5±0.0 ng/mL: p=0.009) and correlated with peripheral and bone marrow matrix metalloproteinase-2 (MMP-2) levels (r= -0.752; p=0.0001 and r= -0.662; p= 0.009, respectively) but not with IL-6 and its soluble receptor. Our results indicate that an imbalance of the OPG/TRANCE ratio in favor of TRANCE occurs in MM supporting the hypothesis that this system could be involved in the pathogenesis of myeloma-induced bone destruction.

Negative Selection of Peripheral Blood Stem Cells to Support Tandem Autologous Transplants in Newly Diagnosed Multiple Myeloma

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In multiple myeloma (MM) the amount of neoplastic cells detectable in peripheral blood stem cells (PBSC) collected after high dose cyclophosphamide and G-CSF or GM-CSF is usually very high. We recently described a procedure whereby PBSC can be efficiently purged of contaminating neoplastic cells by a combination of anti plasma cells monoclonal antibodies. Sixty newly diagnosed MM patients were treated according to a double transplant program and randomized to receive PBSC either unmanipulated or in vitro purged. A median recovery of 75% and 60% of the initially collected CD34+ and CD33+ cells was obtained, so that two transplants were possible. Moreover, this graft manipulation was confirmed to be safe since after each transplant the hematologic and immunologic reconstitutions were rapid and comparable in both arms. No transplant-related mortality was observed. Minimal residual disease (MRD) was evaluated in PBSC before and after in vitro purging as well as in the bone marrow after transplantation by comparing conventional and quantitative PCR. We confirm that in all the analyzed cases the unmanipulated aphereses contained a heavy plasma cell contamination with a median of one neoplastic each 10² normal cells (range 10⁻¹-10⁻⁸). Despite a significant (three to four logs) tumor debulking, all patients remained PCR positive in vivo. At three years, the estimated event free survival (EFS) is 49% in the control arm and 77% in the experimental arm (log rank, p= 0.08) while the estimated OS is 90% and 83% respectively (log rank, p= 0.27). Our results suggest that autologous transplantation can be safely performed in multiple myeloma patients using efficiently purged hematopoietic progenitor cells but confirm the urgent need of innovative protocols for the in vivo eradication of MRD.
**CD107**

**QUANTITATIVE MOLECULAR DETECTION OF IGH REARRANGEMENT IN MULTIPLE MYELOMA: COMPARISON OF TWO INNOVATIVE TECHNIQUES**

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Two innovative quantitative assays for detection of the IgH rearrangement have been employed at diagnosis and in the follow-up of patients affected by multiple myeloma: the GeneScan and real-time PCR. The GeneScan PCR technique allows the amplification of the CD3 region with a JH-consensus fluorescent primer; amplification is followed by a sensitive capillary electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystem). This sequencing apparatus allows the quantitative determination of peak areas; by comparison with the area of different dilutions of a competitor DNA, the quantitation of the testing sample can be performed. Real-time PCR is an innovative method for quantitative molecular determinations; a camera measures the energy emission from a fluorescent dye at every PCR cycle. The Threshold Cycle is inversely correlated with the initial amount of DNA; so, by using an internal control (housekeeping gene) a quantitative determination can be performed. In the lymphoproliferative B-cell disorders, the molecular evaluation of the IgH rearrangement usually employs expensive and time-consuming amplification reactions with patient-specific primers/probes. By using consensus fluorescent primers or CYBR- Green as intercalating dye a satisfying sensitivity and specificity were achieved either by GeneScan or Real-time PCR assays. The two above reported techniques were both employed in 8 patients at diagnosis, on the CD34+ harvests from 15 patients treated by high-dose therapy and in 9 cases after autotransplant. At diagnosis, a cut-off of 1% for the GeneScan PCR and a DDCt of 1 for the real-time PCR were chosen: all tested patients showed the same quantitative results after both molecular analyses. Leukapheresis from all patients resulted still PCR-positive; nevertheless, both molecular assays showed an analogous reduction of this contamination of 1-3 log. After PBSTC, 17% of molecular remissions were found; in two tested patients the progression of malignancy was predicted by the increase of monoclonal IgH rearrangement, detected as an increase of 2 logs by GeneScan PCR and as a relative increase of 6 and 24 times by real-time PCR reactions. These preliminary results show: 1) that consensus primers could be employed instead of patient-specific primers/probes, with a sensitivity sufficient for the prediction of clinical outcome; 2) that the GeneScan PCR and the real-time PCR are comparable, suitable molecular quantitative methods.

**CD108**

**DRUG RESISTANCE MECHANISMS IN MULTIPLE MYELOMA**

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Multiple myeloma is a disease in which conventional protocols of chemotherapy lead to poor results in response to treatment and prognosis and the number of complete remissions remains low, probably because of the presence of an intrinsic or acquired drug resistance. One of the mechanisms responsible for this low sensitivity to therapy is the over-expression of transport proteins involved in multidrug resistance (MDR). The three main proteins involved in this phenomenon are the P-glycoprotein (PGP), related to the classic MDR, the multidrug resistance-related protein (MRP) and the lung resistance protein (LRP), related to atypical MDR. To detect plasma cells we used a monoclonal antibody (BB4 CD138) that binds a molecule expressed at high levels in plasma cells and involved in the cellular adhesion to collagen (Syndecan-1). This antibody allows the detection of plasma cells with results similar to those obtained with the double staining with CD38/CD45. In our study MDR-related proteins were evaluated in bone marrow samples by flow cytometry using a double staining with CD138 and the monoclonal antibody M RK16 (anti-PGP), MRPm6 (anti-MRP) or LRP56 (anti-LRP). We studied M DR-related protein expression in 19 cases of multiple myeloma at onset and in 12 cases at the end of induction therapy (VAD). At onset 9/19 (47%) patients were PGP-(non-overexpressing cases) (MFI=4.7±0.6), and 10/19 (53%) were PGP+, (MFI=10±4). After the therapy (VAD) 4/12 (23%) were PGP- (MFI=3.9±2) and 8/12 (67%) were PGP+ (MFI=19±6), with a significant increase of expression intensity (MFI) but not of the positivity rate. Seven of 19 (37%) cases at onset were MRP- (MFI=1.3±0.6) and 12/19 (63%) were MRP+ (MFI=7±3). After the induction therapy 5/12 (42%) cases were MRP- (MFI=1.6±1) and 7/12 (58%) were MRP+ (MFI=6±3). MRP expression before and after chemotherapy did not show differences either in positivity rates or in MFI. Six of 19 (31%) cases at onset (MFI=6.6±2) and 8/12 (67%) cases after VAD (MFI=9.9±3) overexpressed LRP with a significant increase of positivity rates and intensity of expression (p=0.03). In conclusion, these preliminary data show that at least one of the three MDR-related proteins is frequently overexpressed at onset and that first-line chemotherapy can increase their expression, particularly that of LRP.

**CD109**

**PRESENTING FEATURES AND RISK OF MALIGNANT TRANSFORMATION IN 624 CASES OF MONOCLONAL GAMMOPATHIES OF UNDETERMINED SIGNIFICANCE**

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A total of 624 patients affected by monoclonal gammopathy of undetermined significance (MGUS) were consecutively admitted to our hospitals from January 1988 to October 2000 and were followed with periodic clinical and laboratory examinations. MGUS was diagnosed according to Durie criteria. Median age was 64 (±12) years. Male to female ratio was 1.2. Isotype
was IgG in 67%, IgA in 14.5%, IgM 13%, IgD 0.1%, biclonal MGUS were 2.5%. The light chain was κ in 60% and λ in 40%. At diagnosis the M-protein level was 1720 mg/dL in the IgG group, 990 mg/dL in the IgA group and 1130 in the IgM group. Serum polyclonal Ig reduction was recorded in 95/624 pts (15%). Bence-Jones proteinuria was present in 41 out of 247 evaluable pts (16.5%). The β₂-microglobulin was evaluable in 365 pts and the mean level was 2 μg/L. Marrow aspiration was performed in 47% (295/694) of the patients; the bone marrow plasma cell percentage (BM PC) ranged from 1% to 18%, with a mean value of 4.5% (±3.6). Skeletal X-ray examination was performed in 29.9% (187/624) and osteolytic lesions were found in 5 out of 187 pts (2.6%). Overall 494 out of 624 (79%) pts are evaluable for the follow-up while 130 pts were lost. The mean follow-up was 35 months (1-154). Thirty-two pts developed multiple myeloma and 4 Waldenström’s disease out of 494 pts (7.2%) after a median time of 60 months (3-144) from the diagnosis of MGUS of IgG, IgA and IgM isotype. This group of patients differed from the population of stable MGUS in the following parameters: the initial mean M-component: 2460 mg/dL vs 1720 mg/dL for IgG (P=0.068), 2220 mg/dL vs 990 mg/dL for IgA (P=0.003), 2210 mg/dL vs 1130 for IgM (P=0.002); BM PC 7.5% vs 4.5%. Bence-Jones proteinuria was present in 25% vs 16.5%. No difference was recorded for β₂m globulin values (2.1 vs 2.0). Another small cohort of MGUS patients (23 out of 494 =4.6%) showed a steady and confirmed increase or M-component values (defined as an increase of M-component > of 500 mg/dL in two consecutive controls) but never developed MM after a mean follow-up 92 months (20-120). These patients were characterized by a mean BMPC at diagnosis somewhat higher than controls (5.9% vs 4.5%) and a more frequent reduction of serum polyclonal Ig levels (30% vs 16%). This retrospective analysis points to the prognostic significance of the M-component level, Bence-Jones proteinuria and BMPC percentage at MGUS diagnosis. According to Baldini we show that MGUS evolving to myeloma present higher values of these parameters at diagnosis and we suggest that a closer follow-up is worthwhile in patients presenting with M-protein > 2 g/L for IgG and > 1 g/L for IgA and a BMPC > of 5%.

CO110
PROGNOSTIC VARIABLES FOR MALIGNANT TRANSFORMATION IN 1,104 PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Most patients with monoclonal gammapathy of undetermined significance (MGUS) do not require specific treatment and have a long life span. A subset, however, tends to evolve to lymphoproliferative disease. Since the benign nature of an MGUS is difficult to ascertain and repeated examinations of the patient over long periods are required, it seems important to identify early factors which might predict a malignant transformation. Although MGUS may occur in infections, connective tissue disorders, chronic liver disease, cancer and immunosuppression, whether such conditions may be associated with a higher MGUS tendency to evolve has not been systematically investigated. Our purposes were: (i) to evaluate the natural history of MGUS, (ii) to identify early predictors of evolution, and (iii) to assess whether associated conditions correlate with disease progression. Overall 1,104 consecutive patients with IgG (n=811), IgA (n=114), IgM (n=130), IgD (n=1), and double (n=48) MGUS diagnosed from July 1975 to March 1998 were included in the study. Cumulative survival probability and cumulative probability of transformation into lymphoproliferative disease were calculated by means of the Kaplan Meier estimator. Univariate and multivariate Cox models were used to identify possible predictors of malignant evolution. At diagnosis, a connective tissue disorder, the serologic evidence of hepatitis B virus or hepatitis C virus infection and a solid tumor were present in 0.6%, 7.6% and 61% of patients, respectively. Twenty-three patients received immunosuppression for previous heart (n=14), kidney (n=2) or allogeneic bone marrow (n=7) graft. In 22 cases paraprotein disappearance was registered at a median of 9 (range, 3 to 192) months after first detection. Cumulative transformation probability at 10 and 15 years was 14% and 30%, respectively. At a median follow-up of 65 (range, 12 to 239) months, 64 MGUS cases (5.8%) evolved to multiple myeloma (n=43), extramedullary plasmacytoma (n=1), primary amyloidosis (n=1), Waldenström’s macroglobulinemia (n=12), non-Hodgkin’s lymphoma (n=6) and B-chronic lymphocytic leukemia (n=1). Evolution was recorded in 5 and 3 patients with viral hepatitis and cancer, respectively. No progression was detected in patients with a connective tissue disorder, or who were receiving immunosuppression for a previous graft. At multivariate analysis, > 5% bone marrow plasmacytosis, detectable Bence Jones proteinuria, polyclonal serum immunoglobulin reduction and high erythrocyte sedimentation rate (ESR) were independent factors influencing MGUS transformation. Age, sex, serum β₂-microglobulin, serum albumin, the presence of a double monoclonal protein, connective tissue disorder, viral hepatitis or solid tumor, as well as a previous transplantation, were not associated with a higher incidence of disease evolution. Careful evaluation of bone marrow plasmacytosis, urinary paraprotein, background immunoglobulins, and ESR might help to identify at presentation patients with MGUS requiring stricter monitoring. Further investigations and longer follow-up studies are necessary to reveal all the possible complications related to immune system alterations in MGUS patients.

CO111
ROLE OF BONE DISEASE BIOCHEMICAL MARKERS IN ASYMPTOMATIC MYELOMA IN IDENTIFYING PATIENTS AT HIGH RISK OF PROGRESSION

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Design and Methods. We studied osteocalcin (BGP) and bone alkaline phosphatase (b-AP) as indices of osteoblastic activity, and deoxypyridoline (DPD) as a marker of bone resorption, in fifty-two MM patients, 39 patients with monoclonal gammopa-
Few therapeutic options are presently available for multiple myeloma (MM) patients who relapse after autologous or allogeneic stem cell transplantation, as well as for patients who are refractory to conventional chemotherapy and are not eligible for salvage high-dose therapy. Thalidomide, a glutamic acid derivative with anti-angiogenic properties, has been recently proposed as an effective therapy for patients with advanced refractory disease. From October 1999 to January 2001, 65 patients (46M/19F) from 7 Italian Institutions were treated with thalidomide. Their median age was 63 years (range 35-78); 26 patients relapsed after autologous stem cell transplantation, either single (n = 12) or double (n = 12); 38 patients had disease progression after ≥ 2 lines of conventional chemotherapy, 2 patients relapsed after allotransplant; 1 patient had not received previous treatment. All patients were in stage III, median β2 microglobulin was 2.9 mg/L, median bone marrow plasma cell infiltration was 50%. Thalidomide was initially administered at 100 mg/day; if well tolerated, the dose was to be increased serially by 200 mg every other week to a maximum of 800mg/day. Median administered dose of thalidomide was 400mg/day. WHO grade > II toxic effects were constipation (52%), lethargy (34%), skin rash (11%), peripheral neuropathy (14%) and leukopenia (3%). Sixty patients are presently evaluable for response; of these, 17 (28.3%) showed > 50% reduction in serum or urine M protein concentration and 11 (8.3%) showed > 25% tumor reduction, for a total response rate averaging 46.6%. After 8 months median follow-up, 15/28 patients are alive and progression-free (at 2 to 16 months), 12 patients have relapsed, 1 patient died of pulmonary edema while still in partial remission. Among pre-treatment variables that were analyzed for their potential relationship with tumor response, only the concentration of vascular endothelial growth factor (VEGF) in the conditioned media obtained upon culture of bone marrow plasma cells was statistically significant. In patients who responded favorably to thalidomide plasma cells secreted a significantly lower amount of VEGF than observed in resistant patients (126±45±165 pg/mg vs 227±11±70 pg/mg, p=0.04). These data confirm that thalidomide is active in patients with advanced relapsed/refractory MM and represent the basis for ongoing clinical trials aimed at testing the role of this drug as front line therapy for newly diagnosed disease.
nation of the apheresis products and the minimal residual disease (MRD) were controlled by Gene Scan Analysis after CDR III PCR. After collection of the PBPC the patients were treated with EDAP (etoposide, dexamethasone, Ara-C, cisplatin) regimen and then underwent a double transplantation, the first one with unmanipulated PBPC and the second one (3 months later) with purged or not purged apheresis product. The conditioning regimen for both transplantations was high-dose Melphalan (200 mg/m²). Out of 110 patients 32 were in stage II A and 78 in stage III A. The purging efficacy using a panel of 3, 4 or 5 anti-B monoclonal antibodies was similar (3 log) and the engraftment after the first transplantation (unmanipulated) and the second transplantation (purged) was identical (10 days for 0.5 × 10⁹/L neutrophils, 11 days for 20 × 10⁹/L platelets and 15 vs 17 days for 50 × 10⁹/L platelets). One patient had a transitory graft failure due to reactivation of CMV infection after the second transplant. The treatment-related mortality for all patients was 5%. With PCR analysis of the CDR III and CDR I region, we documented that the immunomagnetic bead B-cell fractions isolated from the apheresis products had an event free survival (EFS) of 20% at 40 months, in comparison to 50% for patients showing a polyclonal or oligoclonal pattern. Complete remission (CR) (Bone marrow, Bence Jones, IF: negative) and partial remission (PR) were obtained in 48% and in 37%, respectively. Examining the whole population, the impact of the purging was favorable: in fact the median EFS for 53 patients transplanted with purged PBPC was 40 months versus 22 months for the patients transplanted with unmanipulated PBPC (p = 0.038). The median overall survival (OS) was not reached for purged patients being 72% at 4 years after the first autograft, versus 48 months for not purged patients (p = 0.048). Our results confirm that a multi-regimen induction and tandem transplantation represents today a favorable therapy for newly diagnosed myeloma patients and that the purging with negative selection can improve the EFS of the patients prolonging the status of CR.

**CO114**

**MELPHALAN 100 mg/m² AND MELPHALAN 200 mg/m² SHOWED SIMILAR CLINICAL OUTCOME IN NEWLY DIAGNOSED MYELOMA PATIENTS**


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Several trials have shown the superior clinical impact of high (usually 200 mg/m², MEL200) and intermediate-dose melphalan (100 mg/m², MEL100) followed by stem cell support versus oral melphalan and prednisone in multiple myeloma patients. The outcome of myeloma patients treated with MEL100 versus those treated with MEL200 is still unclear. Here we describe a retrospective case-match analysis between MEL100 and MEL200 in two groups of patients with similar clinical conditions to investigate their comparative outcomes. Between 1993 and 1999, 81 patients (median age 63) were treated at diagnosis with MEL100 followed by stem cell support. We compared their clinical outcome to a control group of 81 pair mates (median age 50) matched for serum β₂-microglobulin levels and Durie and Salmon clinical stage. The control group conditioning regimen was MEL200 or dose-intensive comparable treatments: 45 patients were treated at diagnosis with single autologous transplantation and 36 with double autologous transplantation. Transplant-related mortality was 4% after MEL100 and 7% after MEL200 (p=0.1). CR was 43% after MEL100, 63% after MEL200 (p =0.1). Median EFS was 30.4 months in the MEL100 group, 32.6 months in the MEL200 group. Median OS was 56.8 months for MEL100 and 52.5 months for MEL200. In this study MEL200 was not significantly superior to MEL100 in term of CR, EFS and OS despite a significant difference of age (63 vs 50, p<0.001). This study supposes that the optimal melphalan dose for high-dose in multiple myeloma is not yet clearly established.

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allogeneic transplantation

CO115
ASSOCIATION OF INTERLEUKIN-1 RECEPTOR ANTAGONIST ALLELE 2 WITH CHRONIC GRAFT-VERSUS-HOST DISEASE

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GVHD is the major complication of hematopoietic stem cell transplant (HSCT) even after extensive HLA-matching of recipient and donor; this seems to suggest that other factors besides, or in association with, HLA-coded differences might have a role in the immunologic host/donor interactions that characterize GVHD. Cytokines are important mediators of aGVHD, affecting both the initial afferent phases and the effector ones leading to cellular damage at the level of target organs; on the other hand, cGVHD has many features typical of a chronic autoimmune disorder. Cytokine gene polymorphisms have been associated with different levels of in vitro cytokine production and with occurrence or severity of several autoimmune disorders; furthermore, previous studies have shown association of IL-10 and TNF-α gene polymorphisms with TRM or aGVHD occurrence in HSCT recipients. Since interleukin-1 may be involved in the initial phases of cytokine dysregulation that occurs in GVHD, we sought to determine whether gene polymorphisms for IL-1p and its receptor antagonist (IL-1Ra) were associated with GVHD. Gene polymorphisms for IL-1RA and IL-1p have been associated with insulin-dependent diabetes, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, and Sjögren syndrome. Fifty-five HSCT patients (48 from sibling donors, 35 MUD transplants), and their donors, were studied. A 249-bp fragment in the V exon of IL-1p gene (nt 3816-4066) was amplified by PCR, and the presence of TaqI restriction site assessed by amplon digestion; a polymorphism in intronic region 2 of IL-1p gene (IL-1RN), consisting of a variable number of an 86-bp tandem repeat up to a pentanucleotide repeat, was amplified by PCR and directly analysed. We found that IL-1p allele 1 (2=240 bp) in the recipient, either in the homozygote or heterozygote form, was associated with a significantly higher incidence of chronic GVHD, but not of aGVHD; on the other hand, IL-1Ra gene polymorphisms were not associated with either acute or chronic GVHD or TRM. However, the combination of the pro-inflammatory cytokine gene polymorphism IL-1Ra allele 2/IL-1p TaqI+ (that has been associated with higher in vitro IL-1p production) showed an incremental significance on cGVHD occurrence and severity. In a multivariate analysis, IL-1Ra polymorphisms remained a strong independent risk factor for cGVHD together with previous aGVHD. Analysis of donor’s polymorphisms also showed that IL-1RA allele 2 was associated with cGVHD occurrence; donor’s IL-1Ra gene polymorphism showed no association with clinical events in the recipient. Determination of IL-1RA polymorphisms may be of help in identifying HSCT recipients at high risk for chronic GVHD, with the aim to design effective preventive therapies and/or to adopt more aggressive therapeutic protocols.

CO116
IN VIVO MODEL OF NOVEL CD20 SUICIDE GENE SYSTEM*

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The use of allogeneic bone marrow transplantation is hampered by the development of graft-versus-host disease (GVHD). Several attempts have been made to genetically manipulate T lymphocytes in order to control GVHD. We recently described a novel suicide gene system based on the transduction of T cells with human CD20 cDNA. CD20 gene modified cells should become susceptible to exposure to monoclonal anti-CD20 Rituximab antibody (Roche). Double positive CD3/CD20 T cells can be rapidly selected by immunoaffinity and show rapid and efficient lysis in vitro upon rituximab and complement addition. A preclinical in vivo model, which would evaluate the possibility of killing CD20 gene modified cells in vivo, is needed in order to further develop this idea in the clinic. We have used a mouse T lymphoma cell line (EL-4) syngeneic with the C57Bl6 mouse strain and have infected this line with the Moloney-derived retroviral vector Pinco carrying the cDNA of the human CD20 antigen under the control of LTR (LTR-CD20-LTR). EL-4 CD20+ express high levels of human CD20 (124000 molecules per cell as measured by Quantibrite assay) and such expression has remained stable for more than a year in continuous culture. Cells were completely lysed in less than three hours exposure to 5 ng/ml Rituximab in presence of 25% human or mouse serum. We have then injected several animals with EL-4 wild type (EL-4 wt) or genetically modified EL-4 CD20+ by either i.p. or i.v. We found that CD20 transduction did not alter tumour formation at various sites or survival by either route of administration. In order to test the efficiency of Rituximab administration in vivo we chose the dose of 1×104 cell per mouse which kills 100% of animals in 4-5 weeks. 150 µg Rituximab i.p., given either as a single dose at day +1 or as a repeated injection twice weekly for four weeks, protected 100% of the animals. We are at the moment evaluating the efficacy of Rituximab treatment in vivo by amplifying the human CD20 molecule by semiquantitative PCR. Our present data show that an in vivo murine model for Rituximab induced killing of CD20 modified T cells is available which will be further developed.

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CO117
DENDRITIC CELLS (DC1 AND DC2) RECOVERY AFTER ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSLANTATION

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In peripheral blood (PB) two subsets of dendritic cells (DC) have been identified: DC type 1, myeloid, are lineage negative, HLA-DR positive and CD11c positive; DC type 2, lymphoid, are lineage negative, HLA-DR positive and CD123 positive. DC1 activate T lymphocytes, while DC2 seems to induce antigen-specific tolerance. We used a 3-colour flow cytometric assay to assess DC1...
and DC2 reconstitution in 14 patients undergoing allogeneic transplantation for hematological malignancies: 8 patients (group 1) received CD34 positive selected PBSC plus 1x 10^7 DC3 positive cells from an HLA identical sibling donor, while 6 patients (group 2) received not manipulated bone marrow stem cells from a matched unrelated donor. A cohort of 23 autologous transplant patients was used as control group. DC were identified in lysed whole blood as lineage negative (CD3, CD16, CD56, CD14, CD19, CD20). HLA-DR positive, CD11c positive (DC1) or CD123 positive (DC2). Prior to the beginning of conditioning regimen the mean number of DC1 and DC2 per microliter was 2.5±0.1 and 2.8±0.2 (group 1) and 2.9±0.5 and 1.6±0.1 (group 2); this numbers were similar to those of autologous patients (2.9±0.1 and 2.8±0.1), but lower than in normal donors (6.0±0.1 and 6.7±0.1, respectively). In both autologic and autologous patients at day 0 and day +7 the mean DC1 and DC2 number was lower than 0.02±0.001. All autologous patients recovered to the pre-transplant number of DC1 and DC2 within day plus 20 from transplant (3.0±0.1 and 2.7±0.1), reaching to normal numbers after 6 months from transplant (6.2±0.1 and 5.7±0.1). In allogeneic patients instead, the mean DC1 and DC2 number was lower at day +20 and day +60 in both groups and recovered to the pre-transplant number at day +90 (1.2±0.1 and 2.0±0.1-group 1; 2.4±0.1 and 2.4±0.1-group 2). One year after transplant mean number of DC1 and DC2 was lower than in normal subjects in group 1 (1.4±0.1 and 1.1±0.1) but similar to normal subjects in group 2 (5.0±0.1 and 3.1±0.1). In conclusion, DC1 and DC2 recovery is markedly delayed following allogeneic transplant (especially in manipulated stem cells transplants) if compared with autologous transplant: this is probably linked to a delay in immune system reconstitution. Studies are in progress to evaluate the relationship between DC reconstitution and graft-versus-host disease.

CO119
IN VITRO KINETIC OF HEMATOPOIETIC AND STROMAL CELL RECONSTITUTION AFTER UNRELATED BLOOD AND BONE MARROW TRANSPLANT

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The kinetic of hematopoietic reconstitution has been studied by in vitro cultures in 50 patients receiving Cord Blood (CB=26) or Bone Marrow (BM=24) transplant from an unrelated donor. The number of cells infused was:

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<th>CB (n=26)</th>
<th>BM (n=24)</th>
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<tr>
<td>NC x10^6/kg</td>
<td>4.3±2.9</td>
<td>36.0±17.4</td>
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<tr>
<td>pWC x10^9/kg</td>
<td>1.6±0.9</td>
<td>9.6±5.5</td>
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<tr>
<td>CFU-GM x10^5/kg</td>
<td>2.4±3.0</td>
<td>8.8±9.7</td>
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<tr>
<td>BFU-E x10^4/kg</td>
<td>1.3±1.0</td>
<td>11.7±9.0</td>
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<td>GEMM x10^5/kg</td>
<td>1.8±1.4</td>
<td>1.8±2.0</td>
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Time to PMN >0.5x10^9/L and to Plts>20x10^11/L for CB and BM transplants were 30.5 vs. 20 days and 58.5 vs. 20 days respectively. The in vitro growth was evaluated at day +20, +35, +60, +100 and thereafter every 3-6 months. Although the number of cells/progenitors infused was significantly different in the in vitro hematopoietic reconstitution after transplant was quite similar in the two groups of patients. In CB recipients the growth of hematopoietic progenitors (CFU) progressively increased by time. In BM recipients the kinetic of in vitro reconstitution was characterised by a variable growth pattern with a high number of colonies in the first control followed by a significant decrease of the CFU number at day +35. At 3 years after transplant, the clonogenic potential in both groups remained at the low limit of the normal range. Stromal cell reconstitution was studied in 13 patients (CB=9, BM=4) and a confluent layer was detected in 7/9 CB and 3/4 BM in cultures established only later than 6 months after transplant. Individual-specific VNTR genetic loci were amplified by PCR to determine the origin of stromal cells after removal of macrophages. Preliminary results show that in CB recipients the fibroblasts were of host origin, whereas a condition of mixed chimism was detected in patients transplantated with BM cells. From the in vitro study we can conclude that: 1) the hematopoietic reconstitution tends to reach a normal pattern by time and seems to be independent from the cell dose infused; 2) the chimism of stromal cells is absent in CB and mixed in BM recipients.
CO120
DETECTION OF ENGRAFTMENT VERSUS AUTOLOGOUS RECOVERY BY
FLOW CYTOMETRIC DETECTION FOLLOWING BONE MARROW
TRANSPLANTATION (BMT) OF β-THALASSEMIC PATIENTS

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Following BMT, the number of circulating blood cells decrease and then increase again as the bone marrow is repopulated by cells of (1) donor origin, in the case engraftment; (2) patient origin, in the case of graft rejection and autologous recovery; or (3) both donor and patient, in the case of mixed chimerism. During this phase, identifying of the circulating cells is usually established through FISH analysis and DNA analysis using polymorphic markers in which the patients and donors have distinct alleles. We propose a novel method to detect engraftment that is based on differential fluorescence intensities of HbF containing erythrocytes (F cells) found in patients are homozygous β-thalassemia (BB) and donors are either non-carrier (NN) or heterozygous carriers (BN). In the non-anemic population, the percentages of F cell vary from less than 1% to around 30%. Erythrocytes of homozygous β-thalassemia major patients contain little or no HbA. Therefore when their peripheral blood is stained with FITC-conjugated anti-HbF monoclonal antibody, the fluorescence intensities of their circulating F cells are much higher than those of donor origin. We define relative fluorescence (RF) as the mean fluorescence intensity of F cell divided by the mean fluorescence intensity of non-F cell. The RFs found in NN and BN samples are significantly lower than those of the patients (NN: 14.6±2.3; BN: 20.7±4.3; BB: 48.4±5.1; p < 0.0001) and there is no overlap in the 82 samples (8 BB, 36 BN and 38 NN) studied. In cases of allogeneic engraftment, the RF of patients' F cells clearly changed from level of the thalassemic patient to that of a BN/NN. When the donor bone marrow was rejected, a population of very fluorescent F-cell reappeared and the RF returned to the value of a BB patient. Analysis of F-cell profile can be done in as little as 3 hours with a flow cytometer and commercially available monoclonal antibody. This method offers a fast and reliable assessment to the engraftment/rejection status of β-thalassemic patients during recovery from BMT.

CO121
FLOW CYTOMETRIC DETECTION OF RED BLOOD CELL ENGRAFTMENT AFTER
ALLOGENEIC BONE MARROW TRANSPLANTATION

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ABO discrepancy between donor and recipient is observed in 20 to 30% of all allogeneic bone marrow transplants (BMT). Currently, the best approach to manage patients with major ABO-incompatible bone marrow transplants is to infuse after BMT red blood cells (RBC) compatible with both recipient and donor blood types. The detection of chimerism by RBC engraftment analysis in ABO incompatible allogeneic bone marrow transplanted patients may help clinicians to choose the best time to perform transfusions with donor RBC group. RBC engraftment analysis is also used to testify stem cell engraftment and to detect mixed chimerism after BMT. We compared the gel test (DiaMed-ID Micro Typing System), which is routinely used for the detection of RBC antigen-antibody reactions, with detection and quantification of RBC subpopulations by flow cytometry. Mixed RBC subpopulations were detected by flow cytometry using RBC indirectly labelled with human antisera directed to the A, B and H RBC antigens and FITC-conjugated anti-human IgG Fab (Ortho Diagnostic). Double populations by mixing RBC antigens from normal donors in mixtures ranging from 0.5 to 100% of RBC were artificially made. By analysing these mixtures with the gel test, we found that the limit of detection of different RBC population was about 20%. By flow cytometry, the measured values allowed the detection of less than 1% of different cells in artificially mixed populations. Blood samples from 12 ABO incompatible allotransplanted patients were evaluable for donor repopulation at one or more points during the first 6 months and then every 6 months after BMT. Gel test revealed mixed red cell chimerism, ranging from 20% to 71%, starting from 40 days after ABO incompatible BMT, whereas flow cytometry was able to detect donor RBC starting from day 20 after BMT. By flow cytometry, we detected RBC mixed chimerism (mean value of donor RBC: 23.5%, range: 6-63%) from day 10 to 45 and RBC full chimerism from day 90 to 120 after BMT in 11 out of 12 patients. In addition, donor RBC reduction during long-term follow-up enabled early detection of relapse in one patient. However, in other cases of malignancy relapse after BMT, flow cytometry still showed full donor RBC population, while marrow cytogenetic analysis showed only autologous metaphases. These finding suggest that flow cytometric detection of mixed chimerism after ABO incompatible BMT: 1) represent a simple, sensitive and unexpensive method to quantitate erythrocyte subpopulations and to follow engraftment; 2) may reveal RBC engraftment earlier than gel test; 3) provides appreciable help to choose the right time to support transplanted patients with donor RBC; 4) may have more general applications for the study of other haemopoietic chimeras.

CO122
TOLERANCE INDUCTION BY HUMAN CD34+ CELLS AND ANTI-CD40L
ANTIBODY PLUS CTLA-4 Ig: PRECLINICAL STUDIES

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We previously demonstrated that subsets of human CD34+ cells rapidly upregulate costimulatory molecules such as CD40 and CD86, and induce allogeneic T cell proliferation in vitro. In this study we addressed the hypothesis of whether allogeneic T cell reactivity to CD34+ cells can be inhibited and T cell tolerance can be induced by blocking CD40/CD40L and/or B7/CD28 pathways. In vitro primary mixed lymphocyte cultures (MLC) were performed with irradiated normal G-CSF-mobilized-CD34+ blood cells obtained by high gradient magnetic separation, or mononuclear cells (MNC) from the same donor, and HLA mismatched allogeneic CD4+ T cells or MNC as responders for six days. Anti-CD40L (CD154) monoclonal antibody (BIOTEN Inc., Cambridge, MA, USA) induced on average 55±20% and 64±20% inhibition of T cell response to CD34+ cells and to MNC, respectively, but...
a higher dose of the antibody was required with CD34+ cells (50 mg/µL) than with MNC (2 mg/µL). T cell alloreactivity was similarly reduced also by CTLA-4 Ig (2 mg/µL): 56±5% inhibition with CD34+ cells, and 62±7% inhibition with MNC, respectively, whereas combination of CD154 and CTLA-4 Ig potently blocked T cells after stimulation with either CD34+ cells (68±8% inhibition) or with MNC (84±10%) in primary MLC. After primary MLC, responder cells were rechallenged with irradiated stimulators from the same donor in secondary MLC to address whether T cell unresponsiveness was achieved. Complete inhibition of secondary T cell response was observed only with responders obtained from cultures with CD34+ cells, or MNC, in the presence of both CD154 and CTLA-4 Ig (on average 89±15% and 82±3% inhibition, respectively). Also, T cell unresponsiveness was antigen-specific since comparable responses to third party stimulators were obtained by anergic or control T cells. Interestingly, addition of IL-2 (50 UI/µL) did not seem to reverse T cell unresponsiveness. Finally, we demonstrated that irradiated CD34+ cells induce potent allogeneic cytotoxic responses in a standard 51Cr release assay. In these experiments addition of CD154 did not affect generation of cytotoxic T cells after stimulation with CD34+ cells, while CTLA-4 Ig alone prevented cytotoxicity. In conclusion, since CD154 and CTLA-4 Ig have a synergistic effect in inducing T cell anergy after stimulation with human purified CD34+ cells in vitro, new strategies for inducing tolerance across HLA barriers may be investigated in allogeneic hematopoietic CD34+ cell transplantation.

The median time of onset of CMV infection was 46 days (range 21–97). In all cases the reactivation of CMV was associated with pancytopenia and in 6 cases it was concomitant with aGVHD occurrence. Clearance of viremia occurred in 8 pts. (57%), only two patients received more than two weeks of therapy. Patients who did not respond to CDV (6/14) were treated with ganciclovir or ganciclovir/foscavir association: three pts. died (2 of IP and 1 of TTP) and CMV-PCR negativity was observed in the other 3. No patient developed renal toxicity. Patients who responded to therapy showed a significantly lower (p=0.038) mean viral load in plasma, whereas pts with high CMV-PCR load had a slow response to therapy and 2 of them developed IP despite a change to ganciclovir/foscavir treatment. In our experience CDV as first-line pre-emptive therapy was effective in 57% of cases; no renal toxicity was observed and the protocol was feasible also in outpatient setting. CDV therapy may represent the first choice in patients with a low CMV-PCR load, while high CMV-PCR load seem not to benefit from this treatment.
CO12
UNRELATED CORD BLOOD TRANSPLANT (CBT) IN PATIENTS WITH HIGH RISK LEUKEMIA: A LONG-TERM FOLLOW-UP

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Cord blood (CB) represents the most recent source of hematopoietic stem cells used in the unrelated transplant setting. On short follow-up, the outcome of CB transplanted patients is similar to that of unrelated bone marrow recipients. We report here the long-term results of patients undergoing an unrelated CB transplant. Characteristics: from July 1995 to April 2001, 35 patients transplanted with high risk leukemia in <2° CR. The prompt availability of cord blood units from Cord Blood Banks allows a fast allogeneic transplant for patients with leukemia at high risk of relapse. Therefore, the search for an unrelated donor should be addressed, at the same time, to the Bone Marrow Donor Registry and Cord Blood Banks.
CO227
HIGH REMISSION RATE AND LOW RISK OF DISEASE RECURRENCE IN MULTIPLE MYELOMA FOLLOWING PBSC ALLOGRAFT

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Allogeneic transplantation in multiple myeloma (MM) has been traditionally associated with an exceedingly high TRM. To reduce mortality and to improve disease response we have developed a protocol with donor G-CSF-primed PBSC instead of marrow. We report here 27 MM patients allografted with PBSC from their HLA-identical sibs. There were 22 males and 5 females. Their median age was 47 yr (range 31-55). Disease characteristics were the following: IgG=14, IgA=6, IqD=1, Bf=5, non-secr=1; stage I=2, stage II=3, stage III=21, plasma-cell leukemia=1; time to transplant = 8 months (3-107). At the time of allotraft, 10 patients were refractory and 2 in progression. Only 4 were in CR and 11 in PR. Most were conditioned with busulfan and melphalan (N=22), and received donor PBSC collected after priming with G-CSF (N=23) or GM-CSF+G-CSF (N=4). The graft contained a median of 7.9 × 10^6/kg CD34+ and 2.2 × 10^9/L PLT on (median) day 12 grade II-IV acute GVHD developed in 8 cases (42%); 4 out of the 14 patients (52%) achieved complete and sustained allogeneic engraftment, the median follow-up being 30 months (range 6-101). In 4 cases (17%) graft rejection was observed within 30 days and it was followed by complete autologous reconstitution. Five patients (21%) died from transplant related complications. Grade II-IV acute GVHD developed in 8 cases (42%). 4 out of the 16 patients at risk (25%) developed chronic GVHD. Conclusions: BMT from well-selected unrelated donors offers results similar to those obtained in transplants using HLA identical family donors. Therefore this type of transplant may be considered as a curative therapeutic approach in patients with thalassemia.

CO228
UNRELATED BONE MARROW TRANSPLANTATION IN THALASSEMIA

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Allogeneic bone marrow transplantation (BMT) from a genotype-identical family donor is an accepted therapeutic option for homozygous β-thalassemia. However only a minority of patients have access to this curative procedure. The aim of this study is to explore the feasibility of matched unrelated transplants in thalassemia and the possibility of reducing the risk of immunological complications with a careful selection of donor/recipient pairs identical for extended haplotypes or parts of them. Methods. Starting in November 1992, 24 patients (age 2-28 years) were enrolled in the study. Marrow donors were chosen after a complete high-resolution molecular study of HLA Class I and Class II antigens. Seventeen out of the 24 donor/recipient pairs were completely identical from HLA A to HLA DRB1 locus; 7 pairs were identical for two extended haplotypes and 9 pairs shared one extended haplotype. Four patients were prepared for transplantation with a drug regimen that included Busulfan (BU) and Cyclophosphamide (CY), whereas the remaining 20 patients were conditioned with BU, Thiotepa and CY. Cyclosporine and short-term Methotrexate were used for graft versus host disease (GVHD) prophylaxis. Results. Fifteen patients (62%) achieved complete and sustained allogeneic engraftment, the median follow-up being 30 months (range 6-101). In 4 cases (17%) graft rejection was observed within 30 days and it was followed by complete autologous reconstitution. Five patients (21%) died from transplant related complications. Grade II-IV acute GVHD developed in 8 cases (42%). 4 out of the 16 patients at risk (25%) developed chronic GVHD. Conclusions: BMT from well-selected unrelated donors offers results similar to those obtained in transplants using HLA identical family donors. Therefore this type of transplant may be considered as a curative therapeutic approach in patients with thalassemia.
wash sample processing of total peripheral blood. Our data showed a rapid reconstitution of CD4+ and CD8+ T cells; CD4/CD8 ratio was persistently inverted, due to the faster reconstitution of CD8+ T cells, showing a spike 60 days after transplant; the recovery of CD16+/CD56+/CD3- NK cells was prompt and showed a consistent overshoot 60 days after transplant. The preliminary results of this study seem to demonstrate that the immunological reconstitution after reduced intensity allogeneic transplant shows a kinetics similar to that observed after autologous PBSC transplant; in particular the prompt recovery of NK cells could have a clinical relevance sustaining an anti-tumor reaction.

chronic lymphocytic leukemia

CD20

CD20 LEVELS DETERMINE THE IN VITRO SUSCEPTIBILITY OF B-CHRONIC LYMPHOCYTIC LEUKEMIA TO RITUXIMAB AND COMPLEMENT: FURTHER REGULATION BY CD55 AND CD59

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Complement dependent cytotoxicity (CDC) is thought to be an important mechanism of action of the anti-CD20 Mab Rituximab (Golay et al. Blood 95:3900). Here we have investigated the sensitivity of freshly isolated cells obtained from 33 B-CLL, 5 PLL and 6 MCL patients to be lysed by Rituximab and complement in vitro. The results showed that in B-CLL and PLL, the levels of CD20, measured by standard immunofluorescence, correlated linearly with the lytic response. The correlation was statistically highly significant (coefficient 0.92, p<0.0001). Correlation was maintained (coefficient 0.90, p<0.0001) when the number of CD20 molecules was measured in 14 B-CLL patients using calibrated beads. Furthermore, the correlation remained linear when 6 MCL patients were included in the same analysis (coefficient 0.91, p<0.0001), suggesting that CD20 levels primarily determine lysis regardless of diagnostic group. The role of the complement inhibitors CD46, CD55 and CD59 was also investigated. All B-CLL and PLL cells expressed the three molecules but at different levels. CD46 was relatively weak on all samples (MFI<100), whereas CD55 and CD59 expression showed high and low variability of expression, respectively (MFI 20-1200 vs. 20-250). Although CD55 and CD59 levels did not permit prediction of complement susceptibility, the functional block of these inhibitors demonstrated that they play an important role in regulating CDC. Thus, lysis of poorly responding B-CLL samples was increased 5-6 fold after blocking both CD55 and CD59, whereas that of high responders was essentially complete in the presence of either anti-CD55 or anti-CD59 alone. These data demonstrate that CD20, CD55 and CD59 are important factors determining the in vitro response to Rituximab and complement and indicate strategies to potentially improve the clinical response to this biologic therapy.

CO131

POST-REMISSIONAL RITUXIMAB FOR THE TREATMENT OF POOR PROGNOSIS CRHONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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In an attempt to reduce residual disease and to prolong duration of response, 6 CLL patients responsive to previous chemotherapy received 4 weekly doses of anti-CD20 MoAb (Rituximab: 375 mg/m²) on an outpatient basis. Median age was 52 years,
median time from CLL diagnosis was 60 months and median number of prior treatments 2. Prior chemotherapy consisted of FAND (F + Ara-C + Novantrone + Desametazone) in 3 cases, CHOP in 1, chlorambucil and prednisone in 1 and Ara-C + cyclophosphamide in 1. According to NCI criteria, the clinical response to chemotherapy was a CR in 2 patients and a PR in 4. The median number of residual PB CD5+/CD20+ lymphocytes prior to Rituximab administration was 208/mm3; the median proportion of BM CD5+/CD20+ lymphocytes was 7% and 4 patients showed residual enlarged nodes (max.Ø: 2.5 cm). Following Rituximab administration, 5 patients achieved a reduction of residual disease in both PB (median rate of reduction: 98%) and BM (median rate of reduction: 66%). No molecular remissions were achieved. A reduction in the enlarged nodes was recorded in 3/4 patients. One patient showed no significant response. After Rituximab administration, 4 patients achieved a reduction of residual disease in both PB (median rate of reduction: 98%) and BM (median rate of reduction: 66%). No molecular remissions were achieved. A reduction in the enlarged nodes was recorded in 3/4 patients. One patient showed no significant response. After Rituximab, a median number of residual PB CD5+/CD20+ lymphocytes of 6×10³/mm3 and a median proportion of BM CD5+/CD20+ lymphocytes of 4% were present. No side effects were observed during MoAb infusion and no infections were recorded in the follow-up. Four weeks after the MoAb administration, 3 patients underwent a successful PBSC mobilization with G-CSF (Lenograstim: 10 mg/kg/day). The median rate of residual CD5+/CD20+ lymphocytes in the aphereses was 1%, 3% and 4%, respectively. During the follow-up, all patient showed a slow increase of PB and BM leukemic cells. However, after a median time of 9 months, only 1 patient showed evidence of a clinical relapse (PB lymphocytes: 5600/mm³). In conclusion, in this small group of previously treated CLL patients the postremissional administration of Rituximab was safe and effective in reducing residual disease. The benefit of Rituximab in the management of CLL needs to be investigated in larger series of CLL patients.

COI32
CLINICAL AND MOLECULAR MONITORING OF MINIMAL RESIDUAL DISEASE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The analysis of MRD has assumed a growing role in the evaluation of tumour contamination of autografts and during molecular follow-up of patients affected by lymphoproliferative diseases otherwise in CR. It has recently been shown that patients with B cell malignancies such as follicular non-Hodgkin's lymphomas or chronic lymphocytic leukemia (CLL) may remain PCR-positive after chemotherapy or transplantation and the persistence of a positive signal has been correlated with an higher frequency of relapse. We report 10 patients affected by CLL with advanced or symptomatic disease requiring therapy. The median age was 54 years (range, 50-68); the M:F ratio was 8:2. The clinical stage, according to Binet was as follows: 1 patient in stage A, 7 in stage B and 2 in stage C. Four were treated with fludarabine (25 mg/m² day 1-3)- cyclophosphamide (300 mg/m² day 1-3)- mitoxantron (10 mg/sm 1° d) chemotherapy (FCM). Six were enrolled in a program including FCM alternated with DHAP, for four-six cycles, PBSC harvest after a cyclophosphamide (4 g/m²) mobilizing chemotherapy, purging ex vivo with four doses of rituximab (375 mg/m²) and autologous bone marrow transplantation (ABMT) after BEAM conditioning. Two patients underwent ABMT with PBSC (harvest 2×10⁶ CD34+ /kg); one patient, failing mobilization, underwent ABMT with BM (harvest 2.3×10⁶ CD34+ /kg). One stage C patient reached a morphological and phenotypic remission, but failed three rounds of BPSC mobilization and the BM harvest yielded only 1.62×10⁶ CD34+/kg. Two other patients are still in treatment. Toxicity was mild, grade 3 neutropenia and thrombocytopenia being only reported in the more aggressive arm; there was no toxic death. The 6 patients treated with high-dose chemotherapy entered in a program of MRD monitoring with PCR method to detect clonal rearrangements of IGH genes by mean of a patient-specific probe, performed serially on BM and PB samples obtained at different time-points after chemotherapy, mobilization/purging and transplantation. Nine out of ten patients achieved CR according to NCI criteria, and the immunophenotype was non-clonal on BM and PB samples in all cases. The PCR assay for MRD in the harvest was negative in only one out of three evaluable pts. Our results, although very preliminary, seems to suggest that molecular follow-up data from CLL patients undergoing high-dose chemotherapy followed by autologous PBSC or BM transplantation are disappointing. Although clinical CR are frequently achieved (9/10 patients), the persistence of a positive PCR test points to the inadequacy of autografting in eradicating CLL clone. However, although PBSC mobilisation is often sub-optimal in CLL patients in terms of CD34+ yield (medium target 2×10⁶ CD34+/kg), a program of high-dose chemotherapy followed by autografting is feasible, well tolerated with a very mild toxicity even after fludarabine-based chemotherapy.

COI33
PROPOSED IMMUNOPHENOTYPE SCORING SYSTEM FOR MATURE B-CELL CHRONIC LYMPHOID LEUKEMIAS: DIAGNOSTIC ROLE AND PROGNOSTIC SIGNIFICANCE IN A COMBINED CLINIC-O-IMMUNOPHENOTYPIC MODEL

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Mature B-cell lymphoid leukemias (MB-LL) are a relatively frequent group of disorders with heterogeneous biology, morphology and clinical behaviour. We examined different variables for their potential value in predicting clinical outcome in 258 consecutive MB-LL patients (M/F: 1:4; median age: 64 yrs, median follow-up 54 mos and 75 deaths), and verified the diagnostic and prognostic role of a simplified immunophenotypic scoring system (MCSS) based on CD5, CD23, and sIg pattern expression. To do this, a variety of clinical and biological parameters (cytomorphology, histology, karyotype and gene rearrangements) were also evaluated. The patients were immunophenotyped at diagnosis for the more frequently tested antigens and a new scoring system was elaborated: dim sIg = 2 points, CD5+ and CD23+/− ≤ 2; CD5± (CD23 interferent) = 0.5, bright sIg = 0; CD5+ and CD23 = 1, CD5 (CD23 interferent) = 0. The proposed scoring system identified four diagnostic clusters (C1, score 3-4: 149 pts; C2, score 2-2.5: 38 pts; C3, score 1-1.6 pts; C4, score ≤ 0.5: 55 pts) characterized by particular clinical features. Typical CLL-like morphology was prevalent in C1 (88% of cases), non-CLL forms (mainly immunocytoima, SLVL and all of the HCL observed) were significantly more frequent in
CD19

EVALUATION OF THE IMMUNOLOGIC SCORING SYSTEMS FOR THE DIAGNOSIS OF B CHRONIC LYMPHOCYTIC LEUKEMIA USING MULTIPARAMETRIC FLOW CYTOMETRY

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The diagnosis of B-CLL is difficult because of the overlap in clinical, biological and morphological features among the various B-cell lymphoproliferative disorders (B-LPD). Moreover since certain markers which were hitherto considered specific are also positive in disorders other than B-CLL, single marker expression is not useful for a correct immunological diagnosis of B-CLL. Two different studies have shown that only the use of 5 immunological markers, as well as sIg and CD22 expression, CD5, CD23 and FM C7 positivities (Matutes et al., 1994) or sIg intensity, CD5, CD23, FM C7 and CD79b expression (Moreau et al., 1997), combined in a scoring system, allow correct distinction between the B-CLL cases, included atypical forms, and the other B-cell diseases. We have evaluated prospectively the diagnostic role of these two scoring systems, by using multiparametric flow cytometry, a lyse-no-wash method on total peripheral blood, and a gating strategy based on CD19+ cells using the following triple combinations of monoclonal antibodies: x/CD19,CD23/CD22/CD19,CD5/CD10/CD19, CD79b/CD38/CD19, FM C7/CD23/CD19, FM C7/CD38/CD19, CD5/CD11c/CD19. Since January 1997 to December 1999, all B-CLL cases diagnosed at our Division (80 patients) have been investigated by this methodology. The results relative to the expression of the five standard markers used in the two scoring systems were as follows: sIg weak expression 76%, CD5 positive 96%, CD23 positive 91%, FM C7 negative 73%, CD22 weak/negative 19%, CD79b negative 100%. Using a cutoff of 4 points or higher, the accuracy of the scoring system was 57.5% (46 patients) if calculated by the method of M atutes and 85% (69 patients) by the method of Moreau. Furthermore, if a cutoff of 3 points or higher was used the accuracy increased to 90% (72 patients) and to 100% (80 patients), respectively. Thus using the M atutes scoring system 10% (8 patients) out of the B-CLL cases were not properly classified. Our data suggest that the use of CD79b instead of CD22 may increase the diagnostic accuracy of the immunological scoring system to discriminate between B-CLL and the other B-LPD.

CD105

CLINICAL SIGNIFICANCE OF SOLUBLE P53 LEVELS IN B-CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) shows variable clinical outcome and some biological parameters may be associated to the clinical features in its prognostic assessment. Reports from the literature have demonstrated that variability in the bcl-2 family of proteins and p53 mutations corresponds to variability of the clinical course of disease and response to therapy. Moreover, it is well known (Cordone et al., Blood, 1998) that p53 protein levels are strictly correlated both with mutations in the gene and with disease progression and poor prognosis. In order to define the exact role of p53 protein in determining the B-CLL heterogeneous clinical activity, we performed p53 soluble (sp53) immunoenzymatic assays on the plasmas of 168 patients (pts), median age 65 years, 88 males and 80 females, all fulfilling the recommended diagnostic criteria with dim sIg and CD5+/CD23+ immunological pattern. Fifty-one pts had low modified Rai stage, 107 intermediate stage and 10 high stage. Fifty pts were treated with chlorambucil at conventional doses and 44 pts received 6 courses of fludarabine monophosphate. With regard to biological parameters, the thresholds of positivity were set at ≥ 3.5 µU/L for sp53 (range 0.1-183.3), >60 µU/L for soluble CD23 (sCD23, range 0-425) and >30% for CD38 (range 1-871) flow cytometric assay. Thirty-nine pts were sp53 positive (39/168). Sp53 levels were significantly correlated both with sCD23 and CD38 percentages (p=0.00001). Almost all (36/39) p53+ pts were within intermediate/high Rai stages (p<0.00001) and 7/9 pts with lymphocyte doubling time (LDT) <12 months were p53 positive. Higher than 2.2 mg/Lβ2-microglobulin was significantly associated with sp53 ≥ 3.5 µU/L (27/38; p=0.006). Furthermore, the presence of three or more intrathoracic/abdominal lymphadenopathies (>3 cm in diameter) and/or splenomegaly was significantly correlated with sp53 levels (24/39; p=0.0005). Twenty out of 39 sp53+ pts were treated with fludarabine and only 5 pts achieved a complete remission (CR) (26.3% vs 73.7%; p=0.06). With regard to clinical outcome, sp53 positive patients showed a significantly shorter progression-free survival (PFS) (6% vs. 62% at 8 years; p<0.00001) and overall survival (OS) (38% vs. 97%; p<0.00001). In multivariate analysis, sp53 levels were confirmed to be an independent prognostic factor with regard to PFS (p=0.003)
together with CD38 expression ($p = 0.002$). Therefore, sp53 levels, detected by an immunoenzymatic method, clearly identify patients in advanced clinical stage, often with intrathoracic/abdominal lymphadenopathies and/or splenomegaly, showing poor response to fludarabine and worse PFS and OS. In conclusion, sp53 and CD38 might be used to further stratify B-CLL pts into different risk classes in order to enucleate candidates for novel therapeutic approaches.

CO136
IMMUNOGLOBULIN HEAVY CHAIN GENE ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL) is a common lymphoproliferative disorder characterized by the accumulation of clonal, mature, small B-lymphocytes co-expressing the various B-cell-associated antigens as well as the CD5 molecule and surface IgM and IgD immunoglobulins. On the basis of these aspects, it has been assumed that B-CLL arise from pre-germinal centre, naïve, CD5-positive subsets B-cells. Analysis of the immunoglobulin (Ig) variable domain genes can provide important informations regarding the ontogenetic stage of neoplastic B-cell populations, tracing the development stage at which neoplastic transformation has taken place and also assigning these cells to their corresponding normal counterparts. In particular, the presence of Ig heavy (IgH) and light (IgL) chain mutated V-region genes is actually considered the hallmark of germinal center (GC) experienced cells and of their descendants. Recent studies revealed that a significant number of B-CLL cases show Ig somatic mutations suggesting that B-CLL may arise from either pre-GC naïve B-cells as well as from GC exposed B-lymphocytes. Moreover, the presence or the absence of somatic mutations seems to predict the natural history of B-CLL. In particular the presence of unmutated Ig genes seems to be associated with a poorer prognosis and with an advantage stage at the onset of the disease. In order to define the status of Ig genes in B-CLL we analyzed the nucleotide sequence of expressed rearranged IgH genes obtained by direct sequencing of RT-PCR products of 29 well characterized B-CLL cases. VH segments involved in Ig rearrangements belong to the VH1 family in 5 cases, to the VH3 family in 19 cases, to the VH4 family in 5 cases and to the VH5 family in 1 case. The most frequent encountered gene was VH3-11 (n=4). Moreover, 14 cases harbored Ig somatic mutations at a significant level (>2%) with a sequence homology compared to the closest germline gene ranging from 88.4% to 96.7%. Although the median follow-up of this group of patients is too short in order to ascribe a prognostic value to the Ig status, patients with unmutated VH genes showed a more aggressive course with much chemotherapy requirements. These data confirm previous observations suggesting that B-CLL may be divided into two biologically distinct forms that originate either from naïve or GC-experienced B-lymphocytes, with probably distinct clinical courses.

CO137
CLINICAL SIGNIFICANCE OF PLASMA VASCULAR ENDOTHELIAL GROWTH FACTOR LEVELS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Recent reports have suggested that angiogenesis may be involved in the pathogenesis of hematological malignancies. VEGF is an important proangiogenic factor and high levels of this protein have been correlated with poor prognosis in patients with solid tumors and acute leukemia; on the contrary, studies on the expression of VEGF in B-CLL have yielded conflicting results. For this reason, we measured plasma VEGF levels in B-CLL samples from 115 untreated patients (pts) and correlated these levels with disease characteristics and prognosis. The patients’ median age was 66 years and M/F ratio was 1.25; according to Rai staging system 35 were stage 0, 44 stage I, 29 stage II, 5 stage III and 2 stage IV. The median survival was 42 months. For the plasma VEGF analysis the cut-off value was set to 60 pg/mL. We first correlated VEGF levels with other clinicobiological parameters of B-CLL such as age, sex, clinical stages (Rai system), β2 microglobulin (β2m), sCD23 (sCD23), lymphocyte doubling time (LDT) and bcl-2 levels. We found that VEGF values <60 pg/mL were only significantly correlated with high β2m and sCD23 values ($p = 0.04$ for both features). We then evaluated the effect of VEGF levels on survival and disease progression; pts with VEGF above 60 pg/mL had better survival, although this difference was not significant. On the contrary, VEGF levels >60 pg/mL were significantly associated with a worse progression free survival ($p=0.005$), as showed below:

VEGF values <60 pg/mL were significantly correlated with a shorter progression free survival ($p=0.001$) within the Rai 0-I subset; on the contrary no correlation was found within the Rai II-IV subset ($p=0.2$). In conclusion our data suggest that VEGF levels are an useful prognostic factor, mainly to identify those patients with early-stage disease who are at risk of disease progression.
MATURATION DEFECTS IN THE DENDRITIC CELL COMPARTMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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A profound dysregulation of the immune system has been described in chronic lymphocytic leukemia (CLL) patients and several studies have reported defects within the effector T-cell compartment. However, very few data are available on the status of professional antigen presenting cells in CLL patients, although dendritic cell (DC) abnormalities have been described in many malignancies as capable of influencing the defective tumor-associated immune response. We performed this study to investigate the phenotypic and functional characteristics of DC in CLL patients. First, a magnetic immuno-depletion kit (MACS, Miltenyi Biotec) was used to directly sort DC precursors from the peripheral blood (PB) of 13 normal controls and 10 untreated CLL patients. In normal samples, sorted cells showed the morphologic and phenotypic features of immature DC, but underwent maturation after a culture period of 48h in the presence of FCS. In addition to the upregulation of CD1a and CD86, they also acquired the expression of CD80, CD83, CD11c and CD40, and high levels of HLA class I and II antigens. Functional- ly, normal DC were capable of stimulating significant allogeneic T-lymphocyte proliferative responses in allo-MLR. In contrast, CLL derived cells showed only partial signs of maturation in vitro. Unlike their normal counterparts, these cells did not increase their size nor did they acquire a dendritic morphology. Despite the expression of CD1a, CD86, CD11c and CD40, in most cases they lacked CD83 and never expressed CD80. Additionally, between 50 and 100% of CD1a positive cells were found to co-express the FasL, a finding not observed in normal donors. CLL DC showed a limited or absent capacity of allo-stimulation. The addition of TNFα to the cultures for 72h failed to induce any sign of differentiation. To further investigate the maturation potential of the DC compartment in CLL, we used GM-CSF and IL-4 to generate DC in vitro from PB monocytes of CLL patients. Although morphologically and functionally mature DC could be obtained in all 5 cases studied, phenotypic analysis at the end of the cultures showed the persistence of a quota of immature DC, expressing CD14, CD1a, CD86, but not CD80 and CD83, and being in some cases HLA-DR negative. This population was never observed in cultures set up from normal donors. We next asked if this impaired maturation capacity could be ascribed to soluble factors produced by neoplastic CLL cells. DC were generated from normal monocytes in Transwell plates with or without the addition of purified CD19+ cells from 4 different CLL samples. Compared to control cultures, the addition of CLL B cells induced about a 35% reduction in the number of DC generated. Variable degrees of inhibition in the expression of CD1a, CD83 and CD80, and in the allo-stimulatory ability were also observed. Experiments are currently ongoing to identify the soluble mediators of this inhibitory effect. In summary, several lines of evidence point to the existence of maturation defects in the DC compartment of CLL patients. Further studies are needed to assess their influence on effector T-cell function in vivo.

EPIDEMIOLOGY OF MYELOFIBROSIS WITH MYELOID METAPLASIA IN ITALY

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Myelofibrosis with myeloid metaplasia (MMM) is a rare myeloproliferative disorder with a scarcely known epidemiology since case histories come from centers of excellence. For this reason, in 1998 the Italian Registry of MMM (RIMM) was born: it prospectively collects the new MMM cases diagnosed and also follows the cases up. The RIMM now involves 680 units of Internal Medicine, Hematology and Oncology, along with Services of Pathology. In 21 months, 362 patients were reported: only 21% of them were from University or Research Hospitals. Clinical cases were assessed with the diagnostic criteria established by the Italian Consensus Conference (1997) and an annual incidence rate of MMM was estimated: 0.45 in 100,000 inhabitants (females: 0.29; males: 0.62). The incidence rate in Italy is therefore lower than expected (males: -7%; females: -20%). The population at diagnosis is made mainly by males (67%) and median age is 70.4 years (range 24-98; 72% >60 years). Twenty-two percent of the patients had a previous diagnosis of myeloproliferative disease, 8% had a cancer and 4% other hematologic disorders; 9% were exposed to non-drug cancerogens, while 6.5% have a family history of lympho-myeloproliferative diseases and 27% of cancer. Patients received their diagnosis in wards of Internal Medicine (45%) or Hematology/Oncology (55%) and reported fatigue (60%), splenic pain (28%), dyspepsia (26%), weight loss (23%), bleeding (13%) and/or fever (11%). The spleen was not enlarged in 8 patients only (5 of whom had had their spleen surgically removed for cancer or trauma), and, on average, the lower margin was 7 cm from the costal edge. Twenty percent of the patients were transfusion-dependent and median Hb value was 10.3 g/dL (range 3.5-21.8). Fourteen percent of the patients had high severity score according to Dupriez and in the follow-up, which is now 475 person-years, 20 patients have died (annual mortality = 5.3). In conclusion, MMM is a rare disease which preferentially occurs in old males, therefore it is diagnosed and managed out of centers of excellence which enroll patient with a lower age (65 versus 72 years) and severity. A collaborative network among centers, such as the RIMM, is expected to detect high incidence areas, characterize subpopulations of patients, improve the quality of health care and support experimental studies.

DIAGNOSTIC AND CLINICAL RELEVANCE OF THE NUMBER OF CIRCULATING CD34-POSITIVE CELLS IN MYELOFIBROSIS WITH MYELOID METAPLASIA

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We investigated the absolute content of CD34+ cells in the peripheral blood of 84 patients with myelofibrosis with myeloid metaplasia (MMM) and 23 patients with other Philadelphia negative (Ph-) chronic myeloproliferative disorders (CMD). In MMM, the median absolute number of circulating CD34+ cells was consistently high (91.6 × 10^6/L; range 0 to 2,460 × 10^6/L). Receiver operating characteristic (ROC) curve analysis showed that 15 × 10^6/L as a decision criterion for CD34+ cells produced an almost complete discrimination between MMM patients out of therapy and other Ph- CMD (positive predictive value: 98.4%, negative predictive value: 85.0%). MMM patients out of therapy with higher numbers of CD34+ cells had a significantly longer disease duration (p=0.019), higher spleen volume index (p=0.014), liver volume (p=0.002), percentage of circulating immature myeloid cells (p=0.020) and of myeloid blasts (p=0.000). When the number of circulating CD34+ cells was correlated with Dupriez risk stratification, the number of CD34+ cells increased significantly from low risk (median 68.1 × 10^6/L), to intermediate risk (median112.8 × 10^6/L) and high risk patients (median 666.1 × 10^6/L) (F=4.95; p=0.009). When CD34+ cells were correlated with a severity score based on both myeloproliferative and myelodepletive characteristics of the disease, only myeloproliferation index was significantly associated with CD34+ cell level (F=5.7; p=0.000). A significantly shorter interval to blast transformation from the time of CD34+ cell analysis (p=0.003) was observed in patients with more than 300 × 10^6/L CD34+ cells. In conclusion, the absolute number of CD34+ circulating cells allows MMM to be distinguished from other Ph- CMD; it is strongly associated with the extent of myeloproliferation and predicts evolution toward blast transformation of MMM patients.

**CO141**

**EXPRESSION OF PRV-1 GENE IN ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA**

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The polycythemia rubra vera – 1 (PRV-1) gene is a novel member of the u-PAR receptor superfamily and is normally expressed in bone marrow precursors but not in circulating granulocytes. The PRV-1 overexpression has been recently demonstrated by Northern blot analysis in granulocyte from patients with polycythemia vera (PV) (Temerinac et al., Blood 2000; 95:2569). In this study we investigated the expression of PRV-1 in granulocytes isolated from 22 normal individuals (10 males and 12 females, median age 48 y, range 22-71) and from patients with PV (n=26; 17 males and 9 females, median age 58 y, range 22-80), essential thrombocythemia (ET) (n=24; 7 males and 17 females, median age 46 y, range 24-80), secondary polycythemia (SP) (n=9; 9 males; median age 60 y, range 34-68) and secondary thrombocythemia (ST) (n=12; 4 males and 8 females; median age 51 y, range 6-73). All PV and ET cases were diagnosed according to the Polycythemia Vera Study Group criteria. Four PV and 6 ET patients were studied at the diagnosis; the median follow-up of the remaining patients was 19 months for PV (range 2-120) and 19 months for ET (range 3-60). PRV-1 expression was evaluated by RT-PCR. We found PRV-1 expression in 24 out of 26 PV patients (92.3%) and in all the ET patients; PRV-1 was undetectable in normal individuals, in all SP cases and in 8 of 9 ST cases evaluated. PRV-1 expression matched endogeneous erythrocyte colony (EECs) formation in 22 (84.6%) PV cases (2 patients were PRV-1 negative/EECs positive and 2 patients were PRV-1 positive/EECs negative) and in 21 (87.5%) ET cases (3 patients were PRV-1 positive/EECs negative). In 30 females (9 PV, 14 ET and 7 ST) clonality analysis on granulocytes and T lymphocytes was performed by HUMARA assay; all PV patients, 13 ET patients and 2 ST patients showed clonal hematopoiesis. In particular the finding of clonal hematopoiesis was associated with PRV-1 expression in all cases but 1; yet, this PRV-1 negative patient (with ST) was aged 73 and had both monoclonal granulocyte and lymphocyte fractions. These data strongly suggest that PRV-1 expression is a novel and reliable tool in the differential diagnosis of primary and secondary thrombocytosis and polycythaemia.

**CO142**

**CLONAL HEMATOPOIESIS AND RISK OF THROMBOSIS IN YOUNG FEMALE PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA**

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Several studies demonstrated a high prevalence of non random X-chromosome inactivation pattern (X-CIP) in essential thrombocythemia (ET). We investigated the incidence of clonal hematopoiesis in myeloid precursors and endogenous erythrocyte colonies (EECs) in ET patients and its correlation with thrombotic manifestations. Clonal analysis of hematopoiesis using X-CIP was carried out in 48 female patients with ET. Median age was 40 years (range 22-65), median platelet count at testing time was 716 × 10^9/L (220-1300). Female patients older than 65 years were excluded to reduce age related skewing. Clonality was assessed on neutrophils, platelets, EECs and bone marrow CD34+ cells. Cytogenetic analysis showed a normal karyotype and there was no evidence of bcr/abl rearrangement in 21 patients. At the time patients were referred for X-CIP analysis, 16 patients received cytoreductive therapy alone, 19 patients received cytoreduction and antiaggregating or anticoagulation therapy, 7 patients received antiaggregating or anticoagulation therapy and 5 patients received no treatment. No treatment information was available for one patient. Thirteen out of 48 patients (27%) developed thrombosis mainly at diagnosis. Eight patients developed splanchnic thrombosis including splenic, portal, mesenteric vein thromboses or Budd-Chiari syndrome, 2 patients developed superficial vein thrombophlebitis, one patient developed axillary vein thrombosis and 2 patients developed recurrent fetal losses with documented placental infarction. Clonal hematopoiesis was found in 20/48 (41.6%), 18 patients had polyclonal hematopoiesis (37.5%), and 10 patients were considered uninterpretable due to constitutive skewing (20.8%). Clonality was confirmed on purified CD34+ subpopulations from bone marrow, documenting that clonality doesn't appear lineage restricted. There were no statistical differences in age at diagnosis, median platelet count at testing time, and length of follow-up. Thrombotic episodes were significantly more frequent in the monoclonal group...
BONE MARROW IN MYELOPROLIFERATIVE DISORDERS SHOWS INCREASED VASCULARITY WHICH IS COMPLETELY REVERSED BY BONE MARROW TRANSPLANTATION

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An increased number of bone marrow vessels has been described both in acute and in chronic leukemias by numerous authors. In our study micro-vascular density (MVD) and the megakaryocyte (MK) compartment of patients with chronic myeloproliferative disorders (MPD) was evaluated on bone marrow trephine biopsies immunostained with anti CD34 (QBEnd) and anti CD61-MoAbs and an APAAP immunohistochemical technique. Samples from patients with chronic myeloid leukemia in chronic phase (CML CP) (n=17), chronic myeloid leukemia in blastic phase (CML BP) (n=8), polycythemia vera (PV) (n=23), essential thrombocythemia (ET) (n=11) and myelofibrosis (MF) (n=10) were compared with normal bone marrow specimens (n=18). In addition the bone marrow biopsies of 10 patients transplanted for CML were analysed at 1 and 12 months after bone marrow transplantation (BMT). A significant increase in MVD and in the number of hot spots (the fields with the highest concentration of vessels) was observed in CML (both in CP and in BP), in ET and in MF. On the contrary, a normal number of vessels was observed in a large number of cases of PV. The number of MKs (evaluated both by routine morphology and immunohistochemistry) was reduced in CML CP and in MF, and increased in CML BP and in ET and in PV. MKs abnormalities seem therefore to be independent from microvessel proliferation.

Finally, ten patients treated with allogeneic bone marrow transplantation showed, at 1 month follow-up, a sharp decrease of MVD (10±4) in comparison to the pre-treatment samples, suggesting that myeloablation could influence the neoangiogenesis observed in these disorders. Interestingly when MVD was re-evaluated at 1 year follow up, there was a further reduction (5±3), even below the normal value, indicating that a defect, also in the endothelial cell compartment, may be present after BMT.

CO144
PIPOBROMAN IS EFFECTIVE AND SAFE IN THE LONG TERM CONTROL OF HIGH-RISK THROMBOCYTHEMIA: RESULTS IN 118 PATIENTS FOLLOWED FOR 10 YEARS

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Background. Essential thrombocythemia (ET) is a myeloproliferative disorder associated with an increased risk of thrombosis. Definition at diagnosis of the risk factors for thromboembolic events is of primary importance for deciding treatments for ET patients. Aims. The purpose of this study was to evaluate the efficacy and safety of pipobroman (PB) in the long term control of high risk essential thrombocythemia patients (HR-ET). Patients were defined at high risk when one of the following characteristics was present at diagnosis: age > 60 years, history of thrombosis or hemorrhage, or platelets > 1 million/m3. Methods. From 1978 to February 2000 (median follow up 10 yrs), 118 untreated patients with HR-ET (M/F 49/69, median age 62 years, range 25-82), diagnosed according to PVSG criteria, were treated with PB. A thrombotic or hemorrhagic event prior to ET was reported by 17 (13%) and 1 pt (<1%), respectively. At diagnosis, thrombotic or hemorrhagic symptoms were present in 4 (3%) and 4 (3%) pts, respectively, whereas 83 pts (70%) were asymptomatic. PB was given at the starting dose of 0.8-1 mg/kg/day until hematologic response. The maintenance dose ranged from 0.3 to 0.6 mg/kg/day according to platelet values. Results. Hematicologic response (PLT<400×10^9/L) was achieved in 91% of pts. During follow-up, platelets values were maintained under 600×10^9/L in 95 pts (80%), and 46 pts (39%) had values under 400×10^9/L. Actuarial survival at 20 yrs was 64%. Thirteen patients had thrombosis during follow up, with an incidence 11.6±1000 p/y and a cumulative risk of 7% and 14% at 5 and 10 yrs, respectively. Acute myeloid leukemia occurred in 3 pts, with an incidence of 2.6±1000 p/y and a 10-year cumulative risk of 3%. Myelofibrosis occurred in 2 pts, with an incidence of 1.7±1000 p/y and a 10-year cumulative risk of 1.7%. Solid
tumors occurred in 7 pts, with an incidence of $6 \times 1000p/y$ and a 10-year cumulative risk of 7%. The age was significantly associated with a higher risk of death ($p=0.00009$) and thrombosis ($p=0.003$), while the duration of PB had no influence. Conclusions: This long-term study demonstrates in a large series of patients with ET at higher risk for thromboembolic events, that pipobroman is an effective drug for the long-term control of the disease. The 10-year risk of thrombosis, solid tumors, acute leukemia and myelofibrosis was 14%, 7%, 3% and 1.7%, respectively. The duration of pipobroman treatment did not correlate with neoplastic events.

Conclusions: This long-term study demonstrates in a large series of patients with ET at higher risk for thromboembolic events, that pipobroman is an effective drug for the long-term control of the disease. The 10-year risk of thrombosis, solid tumors, acute leukemia and myelofibrosis was 14%, 7%, 3% and 1.7%, respectively. The duration of pipobroman treatment did not correlate with neoplastic events.

**myelodysplastic syndromes**

CO145

IDENTIFICATION OF CRYPTIC CYTOGENETIC DEFECTS IN KARYOTYPICALLY NORMAL MYELODYSPlastic SYNDROMES: AN INTERPHASE FISH STUDY

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Karyotype abnormalities along with blast cell percentage and number of peripheral cytopenia are the most important prognostic parameters in myelodysplastic syndrome (MDS) patients. However the overall incidence of cytogenetic defects in MDS is comprised between 60% and 80% with the highest percentage of abnormal karyotypes detected in more advanced MDS FAB subtypes. Therefore a normal cytogenetic pattern is observed in 20-40% of patients, mostly classified as Refractory Anemia (RA) with or without ringed sideroblasts but also as RA with excess of blast (RAEB) and RAEB in transformation (RAEB-t). This fact may be due to the kinetic characteristics of the dysplastic clone, preventing its identification by conventional cytogenetics, or to the involvement of ambiguously coloured chromosome regions in translocations or deletions. From a prognostic point of view not all cytogenetically normal MDS patients have the same favourable outcome. Considering all these data we have decided to apply interphase FISH in chromosomally normal MDS patients in order to unmask minor clonal populations of dysplastic cells, marked by cryptic defects or by point mutations of specific genes. Interphase FISH has been carried out with commercially available cosmid probes specific for the genes most frequently involved in MDS: EGR1, D7S522, INT2, TEL, Rb, TP53, D20S108. In order to detect the cut-off value for every probe, each one has been simultaneously hybridised together with the alphoid sequence specific for the chromosome containing the gene locus probe used. This last and the correspondent centromeric probe were differently labelled in order to be easily recognized. The cut-off values obtained after examining 200 nuclei from normal controls (bone marrow donors) were fixed at: 5-8% for monosomies, 2-3% for trisomies, 6-14% for gene locus deletions and 2-7% for gene duplications. Up to now 37 karyotypically normal MDS patients have been studied. They have been classified as 26 RA and 11 RAEB. The total number of interphase cells examined per patient was 200. FISH has detected single gene defects in 13/37 patients. Gene deletions were discovered in 8 cases (3 RA and 3 RAEB) while gene duplications in 5 (2 RA and 3 RAEB). The percentage of cells carrying the defect unmasked by FISH was comprised between 11% and 35%. We observed that the FISH defect was correlated with age, being more frequently detected in patients over 60 years of age. A disease progression occurred in 9 patients with an abnormal interphase FISH and in 5 with a normal interphase FISH. In conclusion our study shows that chromosomally normal MDS patients may harbour minor clonal populations of dysplastic cells presenting cryptic abnormalities identified by interphase FISH; the disease outcome is predicted by the cytogenetic defect observed and completely similar to that of patients carrying the same defect on conventional cytogenetics.
CLINICAL IMPORTANCE OF INTERPHASE CYTOGENETICS DETECTING OCCULT CHROMOSOME LESIONS IN MYELODYSPLASTIC SYNDROMES WITH NORMAL KARYOTYPE

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Myelodysplastic syndromes (MDS) are acquired clonal stem cell disorders characterized by ineffective hematopoiesis involving one or more hematopoietic cell lineages. MDS are associated with a high risk of evolution into acute myeloid leukemia (AML). The prognosis seems to be influenced by the presence of specific cytogenetic abnormalities which characterize about 30-50% of MDS. Particularly, the presence of a complex karyotype or the appearance of additional abnormalities is associated with disease progression. In spite of this, several cases with normal karyotype evolving into AML are observed. The Wilms' tumor gene (WT1) is a suppressor gene which can be considered a universal molecular marker of acute leukemias since it is overexpressed by leukemic blasts. The quantitative assessment of WT1 by real time PCR allowed us to demonstrate that it is overexpressed in all the cases of acute leukemia at diagnosis and the levels of expression follow the clinical stage of the disease. WT1 transcript gradually decreases during response to treatment and completely disappears when hematologic and molecular remission are achieved. In addition, the reappearance of WT1 transcript is predictive of relapse. In order to assess the significance of WT1 as a marker of MDS and to verify whether its expression correlates with the disease stage, we used a sensitive real time quantitative RT-PCR method to analyze WT1 transcript levels in 36 BM from patients affected by MDS (13 AR, 9 RAEB, 14 RAEB-T) using as normal controls 33 PB and 20 BM obtained from normal donors. The data obtained showed that normal BM and PB samples express minimal amounts of WT1 transcript with a mean value of 119 and 5 WT1 copies every 10000 ABL copies respectively. By contrast, in all BM samples from MDS patients we found the overexpression of WT1. The amount of transcript increased during disease progression from RA to RAEB, RAEB-T with a mean value of 518 every 10000 ABL copies in RA, 2478 in RAEB and 7029 in RAEB-T. In addition we found that higher WT1 levels are often associated with complex karyotypes and cytogenetic abnormalities correlated with poor prognosis. These data demonstrate that WT1 expression level is a tumor marker for preleukemic or leukemic blast cells of MDS. Therefore, monitoring WT1 expression levels will allow detection of MDS progression.

THE PREVALENCE AND CLINICAL RELEVANCE OF P27 AND CYCLIN E IMMUNOREACTIVITY IN MYELODYSPLASTIC SYNDROMES


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Myelodysplastic syndromes (MDS) are a spectrum of diseases characterized by functional impairment of hematopoiesis, eventually progressing to acute leukemia. The cell cycle is tightly regulated by the cyclin and cyclin-dependent kinase complexes. Abnormal expression of p27 and cyclin E is associated with an adverse clinical course in non-Hodgkin's lymphomas and acute myeloid leukemias. Since the expression of p27 and cyclin E in MDS has not been investigated so far, we immunohistochemically evaluated the prevalence and clinical relevance of p27 and cyclin E immunoreactivity (IR) in bone marrow biopsies from 86 patients with myelodysplastic syndromes: 23 RA; 10 RARS; 39 RAEB, 6 RAEB-t; 8 CM ML; M/F 46/40, mean age 66 years (range 38-80).
CO149

Mechanisms leading to cytopenia in hypoplastic MDS and refractory anemia (RA) are still poorly understood. Immunosuppressive treatment may be successful in hypoplastic MDS and RA. By flow cytometry analysis, we studied FAS, FAS-L and intracellular IFN-γ expression on circulating CD34+ cells in hypoplastic MDS. CD34+ and CD4+ cells in 10 hypoplastic MDS and 20 RA patients. By methylcellular IFN-γ expression on circulating CD34+, CD3+ and CD4+ cells expressing IFN-γ, we documented high number of CD4+ cells expressing IFN-γ, after activation with phorbol myristate acetate (PMA)-ionomycin, in hypoplastic MDS and RA patients. CsA treatment of circulating lymphocytes, at a concentration comparable to that achievable in vivo (500 ng/µL), significantly decreased the number of circulating CD4+ cells expressing IFN-γ in patients with hypoplastic MDS (32±3 vs 14±2 without and with CsA; p=0.02) and RA (15±2.5 vs 10±0.5; p=0.002). In hypoplastic MDS and RA patients, in vitro addition of CsA significantly increased circulating (13±3 vs 25±6 without and with CsA and 24±3 vs 24±5, respectively; p<0.05) and marrow CFC colony growth (19±3 vs 27±6 without and with CsA and 25±3 vs 30±5, respectively; p<0.05), but did not increase secondary marrow CFC. We conclude that: 1) lymphocyte-mediated suppression of hematopoiesis is involved in the development of cytopenia and may account for increased apoptosis of hypoplastic MDS and RA progenitors; 2) CsA may improve committed progenitors but not stem cells growth in hypoplastic MDS by reducing lymphocyte-mediated hematopoietic suppression.

CO150

Mechanisms leading to cytopenia in hypoplastic MDS and refractory anemia (RA) are still poorly understood. Immunosuppressive treatment may be successful in hypoplastic MDS and RA. By flow cytometry analysis, we studied FAS, FAS-L and intracellular IFN-γ expression on circulating CD34+, CD3+ and CD4+ cells in 10 hypoplastic MDS and 20 RA patients. By methylcellular IFN-γ expression on circulating CD34+, CD3+ and CD4+ cells expressing IFN-γ, we documented high number of CD4+ cells expressing IFN-γ, after activation with phorbol myristate acetate (PMA)-ionomycin, in hypoplastic MDS and RA patients. CsA treatment of circulating lymphocytes, at a concentration comparable to that achievable in vivo (500 ng/µL), significantly decreased the number of circulating CD4+ cells expressing IFN-γ in patients with hypoplastic MDS (32±3 vs 14±2 without and with CsA; p=0.02) and RA (15±2.5 vs 10±0.5; p=0.002). In hypoplastic MDS and RA patients, in vitro addition of CsA significantly increased circulating (13±3 vs 25±6 without and with CsA and 24±3 vs 24±5, respectively; p<0.05) and marrow CFC colony growth (19±3 vs 27±6 without and with CsA and 25±3 vs 30±5, respectively; p<0.05), but did not increase secondary marrow CFC. We conclude that: 1) lymphocyte-mediated suppression of hematopoiesis is involved in the development of cytopenia and may account for increased apoptosis of hypoplastic MDS and RA progenitors; 2) CsA may improve committed progenitors but not stem cells growth in hypoplastic MDS by reducing lymphocyte-mediated hematopoietic suppression.
out the cytochemical reaction on bone marrow imprints of 10 normal controls, 6 patients with the 5q- syndrome (M/F=2/4; median age=67; range 46-72 years) and 8 patients with other types of MDS, including 3 cases of refractory anemia (RA), 3 cases of RA with ring sideroblasts (RARS) and 2 cases of RA with excess of blasts (RAEB) (M/F=5/3; median age=62; range 54-86 years), at the onset, not previously treated. Three of the 5q- cases presented del(5)(q13q33), one del(5)(q13q31), while in the others the breakpoint was not identified. In patients with other types of MDS the karyotype was normal (3 cases) or showed various anomalies. Employing a Vickers M 86 scanning and integrating microdensitometer the erythroblast optical density (OD) was counted. In the cases with the 5q- a significant decrease in enzyme activity (mean 45.7±1.7) \( (p=0.0001) \) was observed, whereas in pathological erythroblasts of other types of MDS the OD was significantly higher (mean 97.3±4.4) than in normal erythroblasts (mean 75.9±3) \( (p=0.0001) \). These differences were independent of the maturation stages of the erythroblasts, the progenitor growth in vitro and the apoptosis rate, as measured by TUNEL technique; however, the erythroblast proliferative rate, evaluated by MIB-1 immunostaining, tended to be lower in 5q- cases. Also the hypolobulate megakaryocytes observed in the bone marrow imprints of the 5q- syndrome showed a moderately reduced DHFR level if compared with normal megakaryocytes. In conclusion, for the first time a reduced DHFR expression has been demonstrated in 5q- erythroblasts. At present the cause of this reduction is not known (alterations of the cell cycle or gene deletion); our findings, however, suggest a possible role of this enzyme abnormality in the still obscure pathogenesis of the disease.
chronic myeloid leukemia

P0001
LARGE DELETIONS AT THE t(9;22) BREAKPOINT IN CHRONIC MYELOID LEUKEMIA PATIENTS

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The BCR-ABL fusion gene is thought to play a central role in the pathogenesis of CML; the existence of large deletions adjacent to the translocation breakpoint of derivative 9 chromosome has been described. We collected data on the clinical and biological features of 59 chronic phase CML patients to assess the possible relevance of these chromosomal deletions and any possible associations. To identify carriers of deletions on the derivative of chromosome 9 we performed FISH experiments using two BACs covering regions flanking the breakpoint on der(9). These probes were bA17L7 containing the ASS locus proximal to the ABL gene and bA248J22 distal to the BCR gene: they were used in cohybridization labeling with red (Cy3) and green (FluorX). If one or both probes resulted deleted, patients underwent a second screening using a contig of BAC clones spanning chromosomal regions on 9 and 22 to define the precise distal and proximal breakpoints of the deletion. A total of 30 probes of chromosome 9 and 10 for chromosome 22 were used. Deletions on der(9) were observed in 9 (15%) cases; these were different sizes (in terms of megabases) and had different locations of the distal and proximal breakpoints. In patients with deletions, the WBC count at diagnosis was higher than in those without (176 × 10^9/L vs 96 × 10^9/L, p = 0.01). Splenomegaly at onset of CML was present in 8 (89%) patients with deletions and in 13 (26%) without (p = 0.0006). The b3a2 BCR-ABL rearrangement type was found in 8 (89%) patients with and in 23 (46%) patients without deletion (p = 0.02). Two cases of complex Philadelphia translocation and 2 with complex karyotypic abnormalities were observed in patients with deletions (p = 0.001). Sokal’s risk was calculated: 5 (56%) cases with and only 7 (16%) without deletions were included in the high risk category (p=0.01). Eight patients (89%) with deletions did not achieve hematological and cytogenetic response after 1 year of interferon treatment. Our data show that loss of genes adjacent to the translocation breakpoint may determine clinical and biological features of CML. Further analysis is needed to establish whether deletions are associated with worse prognosis.

P0002
INHIBITION OF COLONY FORMATION AND APOPTOSIS IN CHRONIC MYELOID LEUKEMIA CELLS BY FARNESYLTRANSFERASE INHIBITORS IS NOT MEDIATED THROUGH THE FAS/FAS-LIGAND SYSTEM

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The Ras gene products regulate transduction of growth-promoting signals from membrane receptor tyrosine kinases to the nucleus. To induce cell growth and transformation, Ras must move from the cytoplasm to the plasma membrane. Ras must be farnesylated by an enzyme termed farnesyl-protein transferase in order to allow its linking to membrane. Ras activation plays a central role in leukemogenic transformation by BCR/ABL. We have recently documented that Fas-mediated downmodulation of bcr/abl results in decreased proliferation rate of CML bone marrow (BM) progenitors due to apoptosis of clonogenic cells. Recently, inhibitors of farnesyltransferase (FTI) have been used as oral therapeutic agents for the treatment of some types of cancer. We studied the effects of α-hydroxylfarnesylphosphonic acid and manumycin-A (FTI), potent and specific farnesyltransferase inhibitors, on cell and colony growth as well as on induction of apoptosis in CML cell line LAM A and in fresh cells derived from 25 CML patients in chronic phase. In addition, we investigated the expression of Fas and Fas-Ligand (Fas-L) on CD34+ CML cells and their relationship with FTI activity on CML cell growth. By colorimetric MTT and methylcellulose colony forming assays, we documented dose dependent FTI-mediated inhibition of growth in LAM A cells and in 60% of CML, whereas healthy donor cells were only weakly affected by FTI exposure. By flow cytometric detection of apoptosis, we documented that FTI-mediated inhibition of cell growth may be in part related to induction of apoptosis. The addition of Z-DEVD-fmk, an agent that interferes with caspase 3 activity, partially reverted FTI-induced inhibition of cell growth of LAMA cells. Using two-color flow cytometry with FITC-conjugated anti Fas or Fas-L and PE-conjugated anti CD34, performed in the presence of the metalloproteinase inhibitor K8103 (10 mM) to prevent CD95-L shedding, we found that Fas and Fas-L expression was not modified by FTI exposure and that there was no different susceptibility to FTI-mediated inhibition of cell growth based on apoptosis of CML. The addition of Z-DEVD-fmk, an agent that interferes with caspase 3 activity, partially reverted FTI-mediated inhibition of cell growth of LAMA cells. Using two-color flow cytometry with FITC-conjugated anti Fas or Fas-L and PE-conjugated anti CD34, performed in the presence of the metalloproteinase inhibitor K8103 (10 mM) to prevent CD95-L shedding, we found that Fas and Fas-L expression was not modified by FTI exposure and that there was no different susceptibility to FTI-mediated inhibition of cell growth based on apoptosis of CML. We conclude that: 1) FTI may induce selective colony inhibition and apoptosis of CML cells independently from Fas and Fas-L expression on CD34+ CML cells; 2) farnesyltransferase inhibition may be a new promising therapeutic tool in CML.

P0003
ATYPICAL CHRONIC MYELOID LEUKEMIA: A CLINICAL, HEMATOLOGIC AND CYTOGENETIC SURVEY OF 7 CASES

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An overlap syndrome characterized by dysplastic and prolifer-
ative features and the lack of Philadelphia chromosome and bcr/abl rearrangement is described in a minority of cases which otherwise fit the general diagnostic criteria for chronic myeloid leukemia (CML). Although infrequent, these cases deserve particular interest on clinical and biological grounds given the absence of a specific detectable molecular marker of disease and a more aggressive clinical course as compared to classical Ph+ CML. From 1991 to 2001 seven patients were diagnosed at our institution as having atypical chronic myeloid leukemia (aCML) according to the WHO classification of myeloid neoplasms. Bone marrow samples were investigated at cytogenetic and molecular level and were negative for Ph1 and bcr/abl. This figure represents 5.8% of 120 typical Ph+CMLs observed in last 10 years at our institution. All cases were observed in chronic phase at diagnosis. The median age was 67 years (range 45-77). Median leukocyte, platelet and Hb values were 49 × 10^9/L (range 34.3-164), 152 × 10^9/L (68-545) and 11 g/dL (10-15.2), respectively. No patient showed increased marrow fibrosis or absolute or relative monocytosis. A significant degree of myelodysplastic changes mainly consisting of dysgranulopoiesis and dyserythropoiesis was observed in all but two cases. In four patients cytogenetic abnormalities were demonstrated at diagnosis (2 cases with trisomy 21, one with trisomy 8 and one with trisomy 13). In two cases additional chromosomal changes (49, XY,+21, +21, +8 and 47, XY,12p+) were observed at the time of accelerated-blastic transformation. As initial treatment, three patients received alpha-interferon, the remaining four, aged more than 65, were treated with hydroxyurea and/or thrombopoiesis in all cases without a concomitant increase of marrow and peripheral blasts. One patient is alive in chronic phase at 12 months under treatment with hydroxyurea. In our experience FISH has proven very sensitive and specific for detecting the BCR-ABL fusion signal on chromosome 8 and 9, respectively. In one case with cryptic rearrangement, conventional cytogenetic analysis revealed a normal karyotype and in two cases with complex variants the classic Ph chromosome was absent because of additional material on 22. All the CML patients with complex variant translocations had b3a2 rearrangement type while b2a2 was present in the cryptic abnormality case. Sokal’s risk was calculated: two patients were high risk while one case was in intermediate risk group, as was the patient with cryptic gene fusion. The 4 patients with variant translocation did not achieve hematologic or any kind of cytogenetic response after 1 year of interferon treatment. In our experience FISH has proven very useful in detecting and characterizing variant Ph rearrangements; in fact, FISH with dual-color BCR-ABL PACs and BAC probes is a highly efficient tool for studying CML patients, especially Ph-negative patients. It allows the detection of gene fusion, the determination of the type of the translocation, and a quantitative follow-up of the disease. Although the prognostic significance of variant Ph translocations still remains unclear, we can make some considerations as to our own cases; all the cases described in this report were found to be resistant to interferon treatment, and in one patient the chronic phase was very short, with progression to the accelerated and blastic phase in ten months from diagnosis. Further cytogenetic, molecular and clinical studies are needed to specify the real frequency of these events and their correlation with prognosis.
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mal residual disease after allogeneic bone marrow transplant and as an alternative to serial marrow cytogenetic studies for the follow-up of patients treated with interferon. Little is known on the kinetics of bcr-abl transcripts in patients receiving the tyrosine kinase inhibitor STI-571, which has been recently introduced in the treatment of CML. We present the preliminary results of the quantitative analysis of bcr-abl transcripts by a real-time PCR technique in 13 patients treated with STI-571. Eight were in chronic phase (CP) and refractory to interferon treatment and 5 were in accelerated/blastic phase (BP) (4 myeloid, 1 lymphoid). Patients were treated according to the 002 and 003 protocols of the Italian Cooperative Group for the Study of CML. A serial dilution of total RNA from K562 cells was used as a standard for real-time quantification. Results were expressed as ng of K562 total RNA with the same level of bcr/abl expression, and normalized to GAPDH mRNA (bcr/abl normalized Dose, nD) to monitor pre-PCR steps. The sensitivity threshold for bcr/abl mRNA detection was 1 K562 cell (10\(^{-4}\) dilution of standard RNA). Bcr-abl levels were determined in peripheral blood (PB) and/or bone marrow (BM) samples at the onset of treatment, and after 1, 3 and 6 months. Cytogenetic studies were performed before treatment and after 3 and 6 months. Median follow-up of patients was 13 weeks (range 6-25). Before treatment, the mean bcr-abl nD in BM samples was 39.5±22.5, with a non significant difference between patients in CP (76nD) and in BP (109.7nD) (p=0.47). The mean PB bcr-abl nD was significantly higher in patients in BP compared to those in CP (162.8 vs 6.8; p=0.06). The WBC count was also significantly higher in BP (120.3±12.7×10^9/L; p=0.01). Serial measurements of PB bcr-abl levels showed both interpatient and intrapatient variability, with a general trend towards a progressive reduction with time in 6/8 patients treated in CP but in only 1/5 treated in BP. However in CP patients, the comparison between PB levels at baseline and after 1 and 3 months and between BM levels at baseline and after 3 months of STI-571 treatment did not show significant differences. Bcr-abl levels did not correlate with leukocyte count in most cases. A major or complete cytogenetic response was obtained in 0/5 BC patients and in 5/8 CP patients after three month. Three of 5 complete cytogenetic responders had very low levels of transcript (<1,5nD). The BCR/ABL-negative (L428, Hodgkin-derived; Raji, non-Hodgkin lymphoma-derived) or BCR/ABL-positive (NALM-1, CML pre-B cell blast crisis) were studied. The cell lines were quite homogeneous in terms of further molecular alterations involving apoptotic pathways, such as the presence of altered/mutated p53 molecule, or adjunctive chromosomal abnormalities. The presence of ABL amplification was, however, a unique feature of the cell line K-562. Cells were exposed to 10^{-12}/10^{-9} M DT for 48 hours, then the percentage of apoptotic cells was evaluated via flow cytometry or MTT assay. HB-EGF-negative cells were not responsive to DT, irrespective of the presence or the absence of BCR/ABL fusion protein. Among HB-EGF-positive cells, the presence of BCR/ABL fusion protein was associated to resistance to the pro-apoptotic effect of DT (35±14% of apoptotic cells as compared to 80±12% for BCR/ABL-negative cells at 10^{-9} M DT; p<0.01; 5 experiments). DT was, however, normally internalized and persisted inside the cells, suggesting that switching off the activity of the BCR/ABL fusion protein might restore the DT pro-apoptotic capability.

P0007 INTERFERON-\(\alpha\) ENHANCES CO-STIMULATORY MOLECULES AND T8 LYMPHOCYTES IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Interferon-\(\alpha\) (IFN-\(\alpha\)) is a pleiotropic cytokine that has anti-rival, antiproliferative and immunoregulatory functions. In chronic myeloid leukemia (CML), IFN-\(\alpha\) treatment induces long-lasting hematological and cytogenetic control and has several immunological effects including natural killer and T cell cytotoxicity. It was reported that CML cells do not express B7-1 and B7-2 (CD80, CD86) co-stimulatory molecules. In vitro, it may be possible to induce their expression using a combination of cytokines, i.e. GM-CSF/L-4. Addition of IFN-\(\alpha\) to cultures significantly up-regulates their expression and induces the differentiation of antigen presenting cells (APC) which subsequently stimulate a specific anti-leukemic cytotoxic T cell response. We report the case of a patient with CML who achieved a complete cytogenetic response after one year of IFN-\(\alpha\) therapy. At that time, the bone marrow biopsy revealed a relevant lymphocytosis. The immunohistochemical characterization of subsequent marrow biopsies confirmed the persistence of a lymphoid infiltration with a polyclonal T and B component. The immunoph-
nototypic analysis of the PBMC showed a population of cells with high FSC and SSC carrying the CD80 and CD86 molecules and an increased percentage of CD8+ T-lymphocytes. These findings urged us to study the same immunological phenotype in four other IFN-α responsive patients and in ten IFN-α or hydroxyurea (HU) non-responsive patients. The percentage of CD80 and CD86 molecules as that of CD8 T lymphocytes was significantly increased in patients who achieved a complete cytogenetic response in comparison with ten non-responsive patients treated with IFN-α or HU (Table1). As B7-1 and B7-2 molecules, expressed by APC are essential for initiating antigen specific T cell reponses, we have hypothesized that IFN-α therapy produced a T cell mediated response against CML cells through the induction of these co-stimulatory molecules. This case suggests that, in addition to its multiple immunomodulatory effects, IFN-α may also regulate the immune response at the level of APC.

Table 1. Expression of surface marker molecules on isolated PBMC.

<table>
<thead>
<tr>
<th>Mab specificity</th>
<th>Patient treated with IFN-α (N=4)</th>
<th>Patient treated with HU (N=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD80</td>
<td>16±2.7</td>
<td>18±3.8</td>
</tr>
<tr>
<td>CD86</td>
<td>12±3.6</td>
<td>14±2.7</td>
</tr>
<tr>
<td>CD4</td>
<td>16±2.4</td>
<td>17±3.1</td>
</tr>
<tr>
<td>CD8</td>
<td>31±6.6</td>
<td>29±0.6</td>
</tr>
</tbody>
</table>

Results.

In group 1 cellularity was 65 % (60-70). Fibrosis was mild and focal whereas atypical microforms of megakaryocytes were rare. In three cases a moderate histiocytic hyperplasia was seen. In group 2 mean cellularity was 85 % (80-90). Fibrosis was moderate to severe. Atypical microforms of megacaryocytes were present. Conclusions: In our small series, in bone marrow trephine biopsies STI 571 seems to have greater antiproliferative activity and lower fibrogenic effect than interferon.


PO009
THE FUSION PROTEIN BCR/ABL ENABLES RESISTANCE TO APOPTOSIS FOLLOWING INTERNALIZATION OF DIPHTHERIA TOXIN

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In the hematopoietic lineage, heparin-binding EGF-like growth factor (HB-EGF)/diphtheria toxin (DT) receptor is expressed by monocytes (Mo), lymphocytes (PBL) myeloid leukemic cells and a number of myeloid- or lymphoid-derived human cell lines. We have previously shown that primary human neutrophils (PMN) are typically negative for HB-EGF in basal conditions and that recombinant GM-CSF specifically induces HB-EGF in PMN, Mo and ex vivo myeloid leukemic cells. In the present study, we evaluated expression of HB-EGF and sensitivity to DT in ex vivo peripheral myeloid cells from 9 patients with p210-BCR/ABL chronic myeloid leukemia (CML) as well as in the human cell line K-562 (CML myeloid-erythroid blast crisis). Methods included molecular (RT-PCR cloning, Northern blot, flow cytometry, ELISA) and functional (mitogenic activity on BALB/c 3T3 cells, evaluation of apoptotic cells) approaches. The cell line K-562 and the ex vivo CML cells expressed HB-EGF, whereas their normal counterparts were usually HB-EGF-negative, suggesting that the presence of the fusion protein p210 may be involved in activating the HB-EGF promoter, likely through the Ras and MAP kinase pathway. The expression of HB-EGF molecule confers usually sensitivity to the pro-apoptotic effect of DT. By contrast, the p210-BCR/ABL-positive cells as well as the cell line K-562 were not or poorly sensitive to 10-12 to 10-9 M DT pro-apoptotic effect, in spite of the fact that they expressed membrane-bound HB-EGF and DT was internalized. Following stimulation with recombinant GM-CSF or human bladder cancer cell line 5637 conditioned medium, which contains relevant amounts of carcinoma cell-derived GM-CSF, previously HB-EGF-negative, normal myeloid cells upregulated HB-EGF, acquiring a previously lacking sensitivity to DT. Thus, our data provide circumstantial evidence that HB-EGF is one of the molecules induced by the biological activity of the fusion protein BCR/ABL.

The presence of this latter, however, seemed to switch off the pro-apoptotic potential of DT following its internalization through its receptor HB-EGF.
MOLECULAR EVENTS UNDERLYING VARIANT PHILADELPHIA TRANSLOCATIONS IN CHRONIC MYELOID LEUKEMIA

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Variant or masked Ph rearrangements were found in around 5% of CML cases. These rearrangements are represented by simple variants, in which chromosome 22q is translocated to a chromosome other than 9, and complex variants in which, chromosome 9 and 22 rearranged with one or more chromosomes. Moreover, masked Ph can be detected in cases with normal karyotype or rearrangements not involving chromosome 9 and/or 22. No differences in survival and duration of chronic phase between patients with classical and variant Ph translocations were found, whereas a higher frequency of additional structural abnormalities were described in the latter group. We report on cytogenetic and molecular studies of two cases of CML with complex variant Ph translocations involving the short arm of chromosome 6. Cytogenetic analyses showed the following karyotypes:

Pt. #1. 46,XY (19/26)/t(6;9;22)(p24;q34;q11)/t(6;9;22) (p21;q13;q11),+8,i(17)(q10)]/47.XX, t(6;9;22) (p21;q13;q11),+8,i(17)(q10)]/39/42

Pt. #2. M/68, 46, XY [19/26]/45.X, Y,t(6;9;22)j(p24;q34;q11)/7/26

Pt #1 was studied in blastic phase. She refused treatment and died 39 months after diagnosis. Pt #2 was studied under treatment with interferon-α in chronic phase, five months after diagnosis. He was still in chronic phase, at last follow-up, 54 months from diagnosis. FISH was performed with the BCR/ABL1 D-FISH (BCR, 500 Kb, in red and ABL1, 600 Kb in green, Appligene, Oncor), and pairings for chromosomes 6, 9, and 22 (Appligene, Oncor). Breakpoints at 6p were narrowed by applying locus specific probes for the short arm of chromosome 6, associated with an α satellite probe D6Z1 (Appliedgene, Oncor), mapped from telomere to centromere as follows: 409K9-82M9-206F19-136B1-153G14-329A5-108K11-162J16-50J22-1043E3 (red/green signal) was detected at der(22) in both cases. In pt 1, green signals were present on normal 9 and on der(22), whereas red signals (BCR) were present on normal 22 and on der(6). In pt 2 green signals were present on normal 9 and on der(9), whereas red signals (BCR) were present on normal 22 and on der(6). In pt 2 green signals were present on normal 9 and on der(6), while one red signal was detected only on the normal 22. Breakpoints on the short arm of chromosomes 6 were narrowed between pac 162J16 and pac 50J22 in pt 1, and between pac 206F19 and 136B1 in pt 2. Two different cytogenetic mechanisms emerged from the two (t(6;9;22) variant translocations. In pt 1 three chromosome breakpoints resulted into a true complex variant in which the 3' of BCR moved to the 6p21 instead of 9q34. In pt 2 four breakpoints were identified by FISH revealing an additional translocation involving the 9 derived from the BCR-ABL change. Indeed, first a classical t(9;22) occurred, then the der(9)t(9;22) underwent another break, centromeric to ABL1, and generated a reciprocal exchange with 6p. The 3' end of BCR was deleted. Two different loci genes of 6p were involved in the pathogenesis or progression of these cases of Ph-positive CML. The common genomic consequence of the two different mechanisms for complex variant Ph rearrangements was the absence of the reciprocal ABL1-BCR fusion gene.

Acknowledgments: partially supported by A.I.R.C.

PROTEASOME INHIBITORS SHOW A POTENT CYTOTOXIC EFFECT ON CHRONIC MYELOGENOUS LEUKEMIA (CML) BUT NOT ON NORMAL HEMATOPOIETIC PROGENITOR CELLS

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New therapeutic strategies to prevent leukemic cells escape from cytotoxic drugs killing involve the activation of apoptosis. Inhibition of the multicalytic proteasome complex can lead to cell cycle arrest, activation of caspases and ultimately cell death. Thus proteasome inhibitors (PI) may be exploited to modulate the apoptotic process in proliferating cells with little effect on quiescent or terminally differentiated cells. In the present study we tested the effect of a cell permeable proteasome inhibitor with chymotrypsin-like activity (PSI). CFU-GM derived from CD34-enriched bone marrow from CML patients were at least three fold more sensitive to PSI than those derived from normal bone marrows. The mean dose of proteasome inhibitor which induced a 50% inhibition in the growth of CFU-GM was 15 nM for CD34+ cells from patients affected by CML and 50 nM for normal subject, suggesting a more effective killing of leukemic rather than normal progenitors. To further confirm the fact that PSI was not myelotoxic, bone marrow mononuclear cells from 3 different normal donors after treatment with PSI 15 nM were seeded at limiting dilution on the murine stromal cell line M210B4 to evaluate the frequency of the more primitive precursors LTC-IC. The mean LTC-IC frequency in 101 bone marrow mononuclear seeded cells was 154 in 15 nM PSI treated samples versus 184 of the untreated samples. The difference between PSI treated and untreated specimens was statistically not significant (p=0.576). These results indicate that proteasome inhibitors, alone or in association with other cytotoxic agents, have anti-tumor activity against CML, but are not cytotoxic to normal hematopoietic progenitor cells.

FICTION STUDIES IN A PATIENT WITH SIMULTANEOUS PRESENTATION OF CHRONIC MYELOID LEUKEMIA (CML) AND B-CHRONIC LYMPHOID LEUKEMIA

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A 64-year old man presented with slight leukocytosis (38.8 ×10^9/L) and a differential count of 51% neutrophils, 3% eosinophils, 8% basophils, 2% monocytes, 1% blasts, 2% pro-myelocytes, 9% myelocytes, 1% metamyelocytes, 10% lymphocytes, 13% smudge cells. Platelet count was 610×10^9/L. Leukocyte alkaline phosphatase score was 31 (n.v. 40-60). Bone marrow biopsy showed a marked increase of granulopoietic lineage, with normal maturation rate, a slight increase of megakaryocytic lineage, a normal erythropoiesis and a fair nodular infiltration of mature-appearing lymphocytes. Immune surface marker analysis of blood and bone marrow lymphocytes revealed 50% of cells to be CD5, CD19, CD23 positive, Slg (\(\lambda\)) negative and 34% CD5+CD19 (simultaneous staining) positive.

To be CD5, CD19, CD23 positive, Slg (\(\lambda\)) negative and 34% CD5+CD19 (simultaneous staining) positive.

Cytogenetic studies on bone marrow cells showed a 46,XY, t(9;22) karyotype. A diagnosis of chronic myeloid leukemia (CML) with concomitant chronic lymphocytic leukemia (CLL) was established. Further investigations with interphase FISH and FICTON were performed in order to understand the genetic characteristics and the origin of the two hematologic malignancies in this patient. Interphase-FISH, was performed on peripheral blood nuclei with a LSI D13S25 Spectrum Orange and Rb-1 Spectrum Orange (Vysis, Downers Grove, IL). Centromeric probe for chromosome 12 (C-12) was also tested (Oncor, Gaithersburg). FICTON (Fluorescence Immunophenotype and Interphase Cytogenetics as a Tool for Investigation of Neoplasms) was performed as described by Weber Matthiesen et al. (Histochem Cytotechn 1992; 40:171-5). Briefly, cytopsins from mononuclear cells of patient's peripheral blood and bone marrow were incubated with monoclonal antibody for 30 min at room temperature. Anti-CD13 and anti-CD5 (Dako) were used. Staining was performed with a three step technique employing Amca-conjugated polyclonal antibodies (Jackson/Dianoiva, Hamburg, Germany). After immunostaining, the slides were fixed in Carnoy’s fixative (methanol:acetic acid, 3:1) and 1% paraformaldehyde, dehydrated in ethanol series and hybridized overnight with BCR-ABL probe (Vysis, Downers Grove, IL, USA). Immunophenotype and hybridization signals were evaluated simultaneously on a Olympus microscope with triple band filter. Results. Three hundred interphase nuclei were evaluated by FISH: 18% of nuclei showed only one signal corresponding to D13S25 probe, whereas the Rb-1 probe gave two signals, as expected in normal cells. Trisomy 12 was excluded by results obtained with C-12 probe. Double color BCR-ABL probe gave fusion signals on CD13-positive cells, while CD5-positive cells were negative. Conclusions. In this patient concomitant CML and CLL corresponded to two distinct clonal genomic events; the Philadelphia translocation for CML, and a 13q deletion (D13S25) for CLL. Accordingly, only myeloid, CD13-positive cells, showed the BCR-ABL fusion gene, while all the CD5-positive lymphocytes were negative. These results strongly support a dual neoplasia originating from independent targets, one myeloid and one lymphoid precursor, respectively.

Acknowledgments: partially supported by AIRC (Associazione Italiana per la Ricerca sul Cancro).

PO003
MIXED BACKBONE ANTISENSE OLIGONUCLEOTIDE TARGETING R1\(\alpha\) UBUNIT OF PROTEIN KINASE A MAY INDUCE GROWTH INHIBITION AND APOPTOSIS OF HUMAN PRIMARY CHRONIC MYELOID LEUKEMIA CELLS IN VITRO

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Downregulation of protein kinase type I (PKAI) is involved in cell growth inhibition in a broad spectrum of cancer cells in vitro, including those Ras-transformed. It has recently reported a cross talk between Ras and PKA in several types of transformed cells. Ras activation plays a central role in leukemogenic transformation by BCR/ABL. It has also been documented that several phosphorotiorate antisense oligonucleotides targeting the regulatory subunit R1\(\alpha\) of PKAI induce growth inhibition and differentiation of a variety of human cancer cells lines. Recently, it has been shown that antisense oligonucleotides with mixed backbone structure (MBO), containing 2'-O-methylribonucleoside segments at the 5' and 3' ends, exhibit improved pharmacokinetic and bioavailability properties in vivo. We investigated the cytotoxic effect in vitro of a novel mixed backbone 18-mer antisense oligonucleotide (HYB 165) that targets the N-terminal 8-13 codons of the R1\(\alpha\)ubunit of PKAI, on CML LAMA cell line and primary cells from 15 CML. Using the colorimetric MTT assay, we found that HYB 165 was able to induce a dose-dependent growth inhibition in CML LAMA cell line and in 75% of CML at doses ranging between 0.1 and 1 \(\mu\)M after 3 days of incubation (mean percentage (SD of cell growth inhibition: 37(19 and 45(12 at 0.1 and 1 \(\mu\)M, respectively). By methylcellulose colony assay, we documented dose dependent inhibition of colony growth in 80% of CML (mean percentage=SD of colony growth inhibition: 60±20 and 70±12 at 0.1 and 1 \(\mu\)M, respectively). A control PKAI antisense oligonucleotide, designated as HYB 239, only weakly affected CML cells and colony growth. By flow cytometric detection of apoptosis, we documented that the inhibition of CML cell growth mediated by antisense oligonucleotide targeting R1\(\alpha\)ubunit of PKAI may be in part related to induction of apoptosis. These results suggest that: 1) MBO antisense oligonucleotides targeting PKAI induce growth inhibition and apoptosis in primary CML cells in vitro; 2) downregulation of PKAI may be part of new therapeutic strategies in CML.

PO014
A VERY LONG PH+ CHRONIC PHASE (34 YEARS) CHRONIC MYELOID LEUKEMIA IN A PATIENT TREATED WITH CONVENTIONAL CHEMOTHERAPY

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Chronic myeloid leukemia (CML) is a hematological neoplasm that sooner or later progresses from a benign chronic phase (CP) well controlled by chemotherapy to the resistant, accelerated and blastic phases. Using conventional chemotherapy (busulfan and hydroxyurea) in CP, the median duration of survival from diagnosis
is still of the order of 3 to 5 years. Although busulfan or hydroxyurea (HU) induce a morphological remission in the peripheral blood and bone marrow, they do not reduce Ph1+ clones and are unable to avoid transition to terminal phases and to prolong the overall survival. There are few observations regarding some patients having survived from unusual periods of times, i.e > 15 years. Here we describe a very long Ph1+ CML (34 yrs) in a single patient treated with conventional therapy. A male born in 1937, was first seen in 1967 for the evaluation of asymptomatic leucocytosis (WBC count 40,000/µL) and mild splenomegaly; bone marrow showed increased cellularity; no chromosomal investigation was performed at diagnosis, but 5 years later a cytogenetic analysis revealed the presence of Ph1+ chromosome. Treatment with busulfan caused hematological remission (HR), therefore the drug was discontinued in 1989. From 1989 to 1997 the patient’s clinical condition was excellent and routine blood analyses were always normal. In February 1998, 31 years after first presentation, WBC count raised to 35000/µL, cytogenetic study was repeated but no mitoses were available; p210 BCR-ABL determined by qualitative RT-PCR expressed a typical b3a2 transcript. A treatment with low-dose HU caused another HR. In January 2001 the patient’s clinical condition worsened (bone pain); the spleen was enlarged (4 cm from the costal border), the WBC count was 37000/µL. Bone marrow examination excluded blast crisis; FISH analysis detected signals in 70% non-dividing (interphase) cells; quantitative competitive RT-PCR for BCR-ABL showed high levels of BCR-ABL (8×10^4 µg of RNA, ratio BCR-ABL/ABL=80%). Higher doses of HU were administered causing improvement of the patient’s clinical condition. Despite this patient fulfills the clinical diagnostic criteria for CML, his survival time (34 years) is remarkably long and quite rare, even with persistence of Ph1+ cells. We believe that it’s worth thoroughly studying the underlying mechanism playing a role in such unusual cases as ours (immunological factors or unusual and not documented genomic alterations in Ph1+ clone?).

**PO015**

**RARE P190 BCR-ABL CHRONIC MYELOID LEUKEMIA: A CASE REPORT**

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Background: The hallmark of chronic myelogenous leukaemia is the presence of the Philadelphia chromosome; its resultant BCR-ABL gene is generated by alternative genomic breakpoints. The different chimeric proteins are responsible of the considerable heterogeneity both in the disease’s presenting clinical features and in the time taken for evolution to blast crisis. The p190 BCR-ABL (m-bcr breakpoint) is the smallest of these fusion proteins and is generally associated with Ph-positive ALL. In this report we describe a rare case of Ph-positive chronic myelogenous leukemia with p190 BCR-ABL, occurring in a patient with elapsed indolent NHL. Methods. Patient was studied and followed-up by cytogenetic study, molecular biology analyses and clinically. Case report: In 1980, our patient, an 67-years old woman, was admitted because of a severe abdominal pain and subjected to diagnostic screening. Massive splenomegaly and hepatomegaly were found at the clinical examination. Histo-

**PO016**

**AUTOIMMUNE COMPLICATIONS IN A CHRONIC MYELOID LEUKEMIA PATIENT TREATED WITH INTERFERON-α**

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Long term treatment with interferon α (IFN-α) and also β can be complicated by some immunomediates diseases, above all the autoimmune hypothyroidism. The occurence of autoimmune complications in patients with chronic myeloid leukemia undergoing IFN α treatment, as reported by some authors (Talpaz et al., J Clin Oncol 1995), is infrequent: 2% hypothyroidism, 1% hemolitic anemia, 2% connective tissue diseases. There is only one report of severe autoimmune hepatitis in CML patient (Steegmann et al., AM J Hematol 1998). We describe a 55 year old woman with CML treated with IFNα, who developed hypothyroidism and hepatitis, both autoimmune. This patient...
was treated with IFNα at a dosage of $3 \times 10^6$ U per day, initially associated for 6 months with AraCytin (10 days/month), achieving a complete hematologic but only partial cytogenetic response after 6 months. An increase in hepatic enzymes and in TSH level presenting thyroid peroxidase (TPO) antibodies (Abs) appeared after 16 months of treatment. Progressively the patient developed hypothyroidism with high level of TPO Abs and hepatitis with a marked smooth-muscle (SMA) Abs count. No viral markers or LKM and antimitochondrial (AMA) Abs were detected. IFNα was stopped after 24 months and in 2 months hepatic enzymes and thyroid function returned to normal and TPO and SMA Abs had disappeared. Many autoimmune states involve an imbalance of cytokines, which regulate the progression and maintenance of autoimmunity. Probably IFN treatment could be implicated in this dysregulation with involvement of dendritic cells which can initiate autoimmunity. Therefore periodic evaluation of antithyroid, SMA and AMA Abs, thyroid and hepatic functionality is recommended in IFN treated patients.

A small subset of acute leukemias are complex to classify due to coexpression of myeloid and lymphoid markers. To define the biphenotypic acute leukemia a scoring system has been proposed by assigning a different value to the myeloid, B or T lymphoid lineage markers. The clinical behavior and the treatment response of biphenotypic acute leukemia are not clearly defined and there is no agreement about the type of treatment required. The aim of this study is to describe the biological and clinical features, as well as treatment response in a series of biphenotypic acute leukemia observed within the GIMEMA Group. Between January 1992 and December 2000, 14 patients were observed, who fulfilled the EGIL criteria: 9 of these patients showed coexpression of myeloid and B-lymphoid markers, and 5 of myeloid and T-lymphoid markers. The following features were observed at diagnosis: M/F 8/6, median age = 33 years (22-69), WBC = $17.2 \times 10^9$/L (1.4-165), blasts in peripheral blood = 64% (0-90%), bone marrow blasts = 90% (57-100%), WHO performance status 0 = 29%, 1 =50% and 2 =21%. Cytogenetics were available in 13 patients: 5 (38.5%) showed t(9;22), in 2 cases associated to complex karyotypic changes; 3 (23%) cases showed complex chromosome abnormalities without t(9;22) and 5 (38.5%) had a normal karyotype. In two of five Ph+ patients PCR analysis showed BCR/ABL rearrangement with p190 expression. One patient died before treatment; while 4 (31%) received a myeloid-like and 6 (46%) a lymphoid-like therapy, 3 (23%) received a personalized therapy. Of the 13 treated patients, 5 (38.5%) obtained complete remission (CR) and 8 (61.5%) were resistant (5 died of disease progression and the others obtained a CR with a second-line treatment). Four patients received bone marrow transplantation. Relapse was observed in 5 patients after a median time of 4 months from CR (2-8 months). The overall median survival was 10 months, with 3 (21.4%) still surviving with a median follow up of 14 months. The results confirm the severe prognosis of biphenotypic acute leukemia. A high proportion of the patients have the t(9;22). The median survival of patients treated with a myeloid-like therapy was lower with respect to those with a protocol lymphoid-like (10 vs 15 months). Further biological studies are necessary in order to better define the feature and lineage commitment of these rare cases with unfavorable prognosis.
LONG ARM DELETIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

BIOLOGICAL, CLINICAL AND PROGNOSTIC IMPLICATIONS OF CHROMOSOME 6


the GIMEMA Cooperative Study Group

del(6q) occurs in 5-15% of childhood acute lymphoblastic leukemia (ALL) and less frequently in adults (5%). While in children this anomaly depicts a subset of B- or T-ALL not associated to poor outcome, for adults there are too few reports to allow any definitive clinical and biologic correlation. In a consecutive series of 374 adult ALL included in the multicenter GIMEMA 0496 trial and prospectively studied by conventional cytogenetics, we identified 18 cases (5%) with long arm deletions of chromosome 6q. The 18 cases (15 males and 3 females, median age 24.5 (range 0.5-274),]) were divided into: i) del(6q) only (n=6); ii) del(6q) plus other numerical and/or structural abnormalities (n=8); iii) del(6q) and other specific translocations (n=4).

The breakpoints, defined in 17/18 cases, encompassed the 6q21-q23 region in 11, 6q25-q27 in 4 and more centromeric regions in 2. Among del(6q) cases with additional karyotypic aberrations (groups ii+iii), these were of structural and numerical type in 6 instances and of structural type only in the others. Of these, 3 cases showed B-lineage specific changes, namely t(9;22), t(1;19) and t(4;11), and 1 T-lineage specific translocation, namely t(10;14). DNA analysis performed in 13/18 cases demonstrated homozygous and/or hemizygous deletions at the p16 and/or p15 locus in 2/5 cases in group i and in 1/8 cases in group ii, all of which had a T-cell immunophenotype. RT-PCR analysis revealed a BCR-ABL, E2A-PBX1, MLL-AF4 fusion transcript in 3 cases of group iii which also carried the corresponding chromosome translocations. A rearrangement was also detected in 1 case of group ii. The median WBC count of the 18 patients was 14×10⁹/L; patients in group i had higher WBC counts than group ii (median 52×10⁹/L vs 14×10⁹/L). A T-cell phenotype was identified in 6/6 cases in group i and in 3/12 cases in groups ii+iii (p=0.0027). Multidrug resistance (MDR)/P glycoprotein expression tested in 14/18 cases was absent in 11. Overall, 18 patients were treated according to the GIMEMA 0496 and 0597 protocols and analyzed through a centralized handling of the samples at presentation, we have investigated by cytogenetic and/or molecular analysis the t(1;19) and/or E2A-PBX1 gene fusion. We report the clinical, morphological immunophenotypic, karyotypic and molecular features, multidrug resistance (MDR) expression and outcome of 8 patients (3 males and 5 females) with E2A-PBX1 gene fusion (3.9%). The median age was 20.5 years (range 15-58). Four patients showed hepatosplenomegaly, 1 also lymphadenopathy. The white blood cell count ranged between 3.9 and 164.0×10⁹/L (median 8.4). All patients had B-lineage common ALL, the blasts being CD19⁺ and CD10⁺. Cytoplasmatic immunoglobulins (CyIg) were expressed in 5/6 cases tested and the myeloid associated antigens CD13, CD33, CD14 were negative in all cases. CD34 was also negative in all 8 cases. Only 1/8 cases was MDR positive. Molecular analysis showed a E2A-PBX1 gene fusion in all cases. The t(1;19) translocation was present in 4/8 cases tested (3/4 in an unblanced form) and all 4 had additional chromosomal abnormalities. Four cases had at presentation a normal or a failed karyotype; 3/4 studied by interphase fluorescence in situ hybridization (FISH) proved positive for E2A disruption. All patients were treated according to the GIMEMA 0496 and 0597 protocols, and achieved a complete remission (CR). Five patients showed an hematologic relapse after a median of 7 months (range 1-10). One of the 5 patients also suffered a central nervous system relapse. Three patients are presently alive in first CR with a median follow-up of 28 months (10-44); one of them underwent an autologous transplant with peripheral blood stems cells (molecularly negative) after 6 months post-CR. Based on these data, the E2A-PBX1 gene fusion occurs in 3.9% of adult ALL and appears to characterize a subset of relatively young patients with CD10⁺, often CyIg⁺; B-lineage ALL that do not express CD34 and myeloid associated antigens. Patients who carry this abnormality can be induced into CR, but often show an early relapse. These findings suggest that E2A-PBX1⁺ adult ALL represent a sizable subset of patients with distinct biological and clinical features who, similarly to BCR-ABL⁺ and ALL-AF4 cases, should be considered early for eradicative therapeutic strategies.

LONG ARM DELETIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

E2A-PBX1 FUSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA WITH t(1;19) TRANSLOCATION

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The most common translocation that involves the E2A gene, t(1;19)(q23;p13), is reported in 5-6% of pediatric acute lymphoblastic leukemia (ALL). In a consecutive series of 203 adult ALL enrolled in the GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Aduluto) 0496 and 0597 multicenter protocols and analyzed through a centralized handling of the samples at presentation, we have investigated by cytogenetic and/or molecular analysis the t(1;19) and/or E2A-PBX1 gene fusion. We report the clinical, morphological immunophenotypic, karyotypic and molecular features, multidrug resistance (MDR) expression and outcome of 8 patients (3 males and 5 females) with E2A-PBX1 gene fusion (3.9%). The median age was 20.5 years (range 15-58). Four patients showed hepatosplenomegaly, 1 also lymphadenopathy. The white blood cell count ranged between 3.9 and 164.0×10⁹/L (median 8.4). All patients had B-lineage common ALL, the blasts being CD19⁺ and CD10⁺. Cytoplasmatic immunoglobulins (CyIg) were expressed in 5/6 cases tested and the myeloid associated antigens CD13, CD33, CD14 were negative in all cases. CD34 was also negative in all 8 cases. Only 1/8 cases was MDR positive. Molecular analysis showed a E2A-PBX1 gene fusion in all cases. The t(1;19) translocation was present in 4/8 cases tested (3/4 in an unblanced form) and all 4 had additional chromosomal abnormalities. Four cases had at presentation a normal or a failed karyotype; 3/4 studied by interphase fluorescence in situ hybridization (FISH) proved positive for E2A disruption. All patients were treated according to the GIMEMA 0496 and 0597 protocols, and achieved a complete remission (CR). Five patients showed an hematologic relapse after a median of 7 months (range 1-10). One of the 5 patients also suffered a central nervous system relapse. Three patients are presently alive in first CR with a median follow-up of 28 months (10-44); one of them underwent an autologous transplant with peripheral blood stems cells (molecularly negative) after 6 months post-CR. Based on these data, the E2A-PBX1 gene fusion occurs in 3.9% of adult ALL and appears to characterize a subset of relatively young patients with CD10⁺, often CyIg⁺; B-lineage ALL that do not express CD34 and myeloid associated antigens. Patients who carry this abnormality can be induced into CR, but often show an early relapse. These findings suggest that E2A-PBX1⁺ adult ALL represent a sizable subset of patients with distinct biological and clinical features who, similarly to BCR-ABL⁺ and ALL-AF4 cases, should be considered early for eradicative therapeutic strategies.
PO020
MITOXANTRONE IN THE INDUCTION TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA. GIMEMA PHASE II PILOT STUDY

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Mitoxantrone (MTZ) is a derivative of dihydroxyantracenedione with a reported activity in the induction phase of AML and in the treatment of CML blastic phase and lymphomas, both as single agent and in combination. Limited information has been reported on the treatment of ALL, in association with the classic drugs vincristine (VCR) and cytosine arabinoside (Ara-C), indicating the possibility of obtaining high CR rate with acceptable toxicity also at high doses (80 mg/m²) (Weiss et al.1996). Based on these experiences, in 1997 GIMEMA cooperative group, in collaboration with Wyeth Lederle, started a pilot phase II study with the aim of verifying feasibility and activity of an induction regimen including a single MTZ dose of 25 mg/m² repeated 3 times during a 36-day period, in association with VCR+PDN+ASP. Between January 1999 and May 2000 a total of 26 patients – 17 males and 9 females, median age 40 years (min 15.6 - max 57.3) have been enrolled by 6 GIMEMA Centers. Median WBC count at diagnosis was 14×10³/L (min 1.6 - max 201), immunophenotype B in 19 and T in 5 cases, with 2 patients classified as biphenotypic leukemia. Ph chromosome has been identified in 6 cases, and t(4;11) in 2. Out of 26 patients eligible and evaluable for response (2 off study before completion of induction for toxicity), 16 (67%) obtained CR (68.4% of Ph negative cases), 3 died during induction and 5 were resistant. A total of 4 cases (including 2 above reported who went off study) did not completed the induction treatment for thromboses (2) and hepatic toxicity (2). One of them was in CR at the time of going off study. Three patients had FOU and two had a documented infective episode: one bacterial (gram neg) pneumonia and one fungal disseminated infection. Both patients died during induction. Out of 16 CR patients, 6 relapsed after a median time of 6 months (min 2 - max 14) and 1 died in CR at 3 months after allograft. To date, 9 patients (56%) are in continuous CR with a median follow up of 13 months (min 9 - max 21). In conclusion: 1) the low number of patients registered does not allow any comparison with results of other trials. The overall CR rate, although not very high, may be considered in the framework of results obtained by larger cooperative GIMEMA ALL trials and confirms the activity of the drug combination; 2) the overall drug-related toxicity is relatively low, with only 2 documented infective episodes. The four episodes causing induction therapy discontinuation occurred during the period of asparaginase administration; 3) the percentage of patients in continuous CR (56%) seems encouraging even if a longer follow up is needed to confirm these data and other larger trials are needed to define the role of MTZ in the front-line treatment of ALL.

PO021
PULMONARY INFILTRATES IN ADULT ACUTE MYELOID LEUKEMIA

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Leukemic infiltration of the lungs may occur in patients with acute myeloid leukemia (AML) in about 20-30% of cases, but it is usually only microscopic, only rarely it is of clinical or radiographic significance and it is invariably associated with hyperleukocytosis, as a consequence of vascular rupture, caused by leukocyte thrombi, or diapedesis through lung vessels, and subsequent infiltration of parenchyma. We report the imaging features of pulmonary leukemic infiltrates in AML without hyperleukocytosis and low percentage of peripheral blasts. Four patients with AML at onset or relapse of disease, were hospitalized in our clinic, presenting with fever, dyspnoea, cough and/or blood gas analysis modifications. They were investigated with standard radiography and high resolution computed tomography (HRCT) of the chest. Routine microbiological tests on the blood and bronchoalveolar lavage (BAL), both by cultural and molecular methods, for bacterial, viral or fungal agents were negative. Of note, none of our patients demonstrated hyperleukocytosis (leukocytes counts ranging from 2500 to 15000 leucocytes/per cubic millimeter). In all patients leukemia manifested predominantly as pulmonary infiltrates. There was no evidence of any other extramedullary leukemic localisation. Standard chest radiography, negative for parenchymal alterations in one patient, showed either a multifocal air space disease or a bronchopneumonia pattern, with pleural effusions, in the remaining three. HRCT scans revealed pulmonary infiltrates with alveolar, interstitial, mixed and peribronchial/perivascular patterns in all patients. Histologic and immunohistochemical analysis of lung biopsy samples showed the presence of pulmonary leukemic infiltrates in these patients. In conclusion the present study provides the first description of the spectrum of HRCT imaging findings in AML patients with pulmonary leukemia. Leukemic infiltrates must be included in the differential diagnosis of the possible causes of clinically evident pulmonary infiltrates in AML patients with low/normal peripheral leukocyte/blast count even when standard chest radiography is negative. HRCT should be performed to complement standard radiographic findings to guide fine needle biopsy, BAL or surgical procedures, and may help in the early identification of pulmonary leukemia with possibly relevant clinical consequences.

PO022
ARSENIC TRIOXIDE THERAPY FOR RELAPSED ACUTE PROMYELOCYTIC LEUKEMIA: AN USEFUL BRIDGE TO TRANSPLANTATION

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The addition of ATRA to chemotherapy has marked a major advance in the treatment of acute promyelocytic leukemia (APL),
significantly improving the remission rate and overall survival. Unfortunately, ATRA does not appear able to maintain patients (pts) in remission and, despite the overall good prognosis, relapses still occur in about 30% of cases. Most pts are usually induced into complete remission (CR) with chemotherapy, since those who relapse while taking ATRA as maintenance therapy rarely obtain a second CR with ATRA alone. Once second CR is achieved, both autologous and allogeneic stem cell transplantation may be useful for reducing relapse by virtue of high dose conditioning regimens and graft-versus-leukemia effect. However, transplantation results may be hampered by toxicities of the previous intensive chemotherapy, since transplant-related morbidity still remains a major problem. Arsenic trioxide (ATO) has proven effective in the treatment of relapsed APL. This drug can induce partial differentiation and apoptosis of APL cells through degradation of wild type PML and PML/RARα chimeric proteins and possible anti-mitochondrial effect. On the assumption that ATO may cause less complications than myelosuppressive chemotherapy, we treated relapsed APL patients with ATO before stem cell transplantation planned in second CR. Patients and methods. Seven patients, M/F 2/5, median age 53 (21-71) entered the study. Six pts were in first untreated relapse, one in second relapse refractory to chemotherapy plus ATRA. The median duration of first CR was 15 mo. At time of relapse all pts were taking intermittent ATRA as maintenance. Two pts suffered also from extramedullary relapse (CNS, lymphnodes, auditory canal, skin). ATO was delivered at the dose of 10 mg/day diluted in 500 mL normal saline over 2 hrs until CR. Treatment with ATO is safe in this report, 5 pts are in CCR after a median follow-up of 7 months.

Results. Seven patients, M/F 4/3, were designed for autologous bone marrow transplantation (1 MUD, 1 genoidentical) within 2 months from CR. The patient in third CR, aged 71 yrs, refused either chemotherapy or transplantation procedures and relapsed after 7 months in spite of maintenance with monthly courses of ATO. The remaining 3 pts, all RT-PCR negative for PML/RARα, were designed to autologous PBSC transplantation after mobilization with intermediate-dose ARA-C, Mitoxantrone and G-CSF. Two were successfully autotransplanted after mobilizing 5 and 5.2 ×10^10/kg CD34+ cells, one failed the first harvesting attempt. At the time of this report, 5 pts are in CCR after a median follow-up of 7 months from second remission. Conclusions: Treatment with ATO is safe and effective in relapsed APL and may constitute an useful alternative to second CR with ATRA alone. Once second CR is achieved, both autologous and allogeneic stem cell transplantation may be useful for reducing relapse by virtue of high dose conditioning regimens and graft-versus-leukemia effect. However, transplantation results may be hampered by toxicities of the previous intensive chemotherapy, since transplant-related morbidity still remains a major problem. Arsenic trioxide (ATO) has proven effective in the treatment of relapsed APL. This drug can induce partial differentiation and apoptosis of APL cells through degradation of wild type PML and PML/RARα chimeric proteins and possible anti-mitochondrial effect. On the assumption that ATO may cause less complications than myelosuppressive chemotherapy, we treated relapsed APL patients with ATO before stem cell transplantation planned in second CR. Patients and methods. Seven patients, M/F 2/5, median age 53 (21-71) entered the study. Six pts were in first untreated relapse, one in second relapse refractory to chemotherapy plus ATRA. The median duration of first CR was 15 mo. At time of relapse all pts were taking intermittent ATRA as maintenance. Two pts suffered also from extramedullary relapse (CNS, lymphnodes, auditory canal, skin). ATO was delivered at the dose of 10 mg/day diluted in 500 mL normal saline over 2 hrs until CR. Treatment with ATO is safe in this report, 5 pts are in CCR after a median follow-up of 7 months.
elderly patients, selected on epidemiological basis by registra-
tion in the GIMEMA archive, were evaluated to compare the
impact on survival of aggressive induction chemotherapy versus
conservative non aggressive treatment. One thousand and five
patients with AML aged >60 years, recorded between July-1992
and December 1997 in the GIMEMA archive were analyzed con-
cerning type of treatment, risk factors at diagnosis and response
to treatment. Six hundred and twenty-one (61.8%) patients
received an aggressive induction therapy (group A), while 384
(38.2%) received a conservative treatment (group B). No differ-
ence was observed between the 2 groups concerning FAB sub-
types distribution, fever or documented infection, liver disfunc-
tion, renal function, haemorrhagic syndrome at onset; there
was an imbalance concerning: median age (67 vs. 73 yrs -
<0.0001), WHO performance status (PS) (stage II-IV =45.25 vs.
60.9% - p<0.0001), previous myelodysplastic phase (3.5% vs.
10.4% - p<0.0001), WBC cell count (3.5 vs. 2.2 ×10^9/L -
<0.0001). Overall median survival was respectively of 7 (0-85)
vs. 5 (0-253) months in group A and B (p=0.0001), with a sur-
vival rate at 12 months of 31.6% and 21.3 months respect-
vatively. At multivariate Cox model analysis the following factors
diagnosis were associated to a significantly reduced survival:
age >71 yrs (RR=1.27; 95% CI=1.07-1.50); PS 2-4 (RR=1.44;
95% CI=1.24-1.68); WBC count >50.000 (RR=1.37; 95%
CI=1.06-1.75); treatment requiring heart dysfunction (RR=1.26;
95%CI=1.05-1.50). No difference in survival was associated with
non aggressive treatment (RR=1.1; 95%CI=0.94-1.32). Despite
an obvious selection of elderly and worse prognosis patients in
group B, the difference in survival between aggressively or non
aggressively treated patients is marginal. The multivariate analy-
thesis did not demonstrate a significant survival benefit in aggres-
sively treated patients; furthermore the longer duration of hos-
pital stay in these patients (37 vs. 16 days - <0.0001) pointed
out a worse quality of life of such patients.

PO025
HYDROXURAREA BEFORE INTENSIVE CHEMOTHERAPY FOR ADULT AML:
THE GIMEMA GROUP EXPERIENCE

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During the last 2 years, GIMEMA A treatment for adult (15-60
years) AML patients included a 3-drug induction cycle with DNR
(50 mg/m^2 d 1, 3, 5), cytarabine (100 mg/m^2 d 1-10), etosporine
(100 mg/m^2 d 1-5) followed by an intensive consolidation with
cytarabine (500 mg/m^2 q12h d1-6) and the same anthracy-
cline as in induction on d 4-6. Following consolidation, eligible
pts (age <45 or 55 yrs) with an HLA compatible sibling had to
be autografted, the others, had to be autografted with autologous
peripheral stem cell (PSC) collected during recovery from con-
solidation. BM and PB samples at diagnosis were centralized
according to a national GIMEMA original study planned with
the aim to accurately evaluate biological characteristics at diag-
nosis and to identify genetic alterations with prognostic rele-
ance and to follow up cases monitoring minimal disease dur-
ing remission. To allow the adequate collection and sending of
samples before starting intensive chemotherapy, all patients
received a 5-day pretreatment consisting of hydroxurea (HU)
at the dosage of 2 g/m^2/day, also effective for debulking of dis-
ease. A preliminary analysis has been performed on patients
enrolled between October 1998 and January 2001, in particular
with regard to the impact of HU cytoreduction on response. Out
of 209 cases evaluable for response, 141 (67%) obtained CR
after the first induction cycle. The response was associated with
the WBC count at diagnosis: in patients with WBC <50 ×10^9/L,
112 out of 151 patients achieved CR (74%); versus 29 out of 58
in the others (50%), p=0.001). The WBC count after the HU pre-
treatment decreased to less than 50 ×10^9/L in 32 out of 58 cas-
es. The CR rate in the latter group was 56%, versus 42% in
patients who remained with a WBC count higher than 50 ×10^9/L
(p=N.S.). Conclusions: overall CR rate of the GIMEMA A treatment
for adult AML (LAM99P) is not different from results obtained
in the previous AML10 EORTG GIMEMA A trial in patients receiv-
ing the same treatment (standard arm), who did not regularly
received HU pretreatment; delay of starting the intensive
chemotherapy to complete all biological sampling needed for
centralization of diagnosis does not seem to have any negative
impact on the induction response to the treatment either in
patients with a lower (<50 ×10^9/L) and an higher (>50 ×10^9/L)
WBC count at diagnosis.

PO026
ACUTE MYELOID LEUKEMIA SECONDARY TO ESSENTIAL
THROMBOCYTHEMIA: DIFFERENT CYTOGENETIC DEFECT IN PATIENTS
TREATED WITH PIPOBROMAN AND HYDROXUREA

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A transformation of ET in AML preceeded or not by a myelodys-
plasia (MDS) mainly happens in patients treated with radi-
ophosphorous or alkylating agents, especially Busulfan. In 1998,
however, it was raised concern even about the long-term safety
of HU: 13% of HU treated patients developed MDS/AML, showing a 17p-,
associated with vaculated neutrophils pre-
senting the Pelger-Huet anomaly. The leukemogenic risk of PI,
another drug used and well tolerated by ET patients, has never
been fully defined. We report on 171 ET patients treated at our
Institution from 1985 to 1996, and monitored until December
2000. Thrombocytosis was well controlled by PI in 122 patients
and by HU in 23. Twenty-six patients received no treatment. After
a median follow-up of 104 months a transformation in AML
occurred in 7 patients (4 received HU and 3 PI) in MDS in 2
patients both treated with PI. A 17p- was the commonest cyto-
genetic defect in 3 HU treated patients (only one signal due to
the TP53 DNA probe), while the fourth carried a 17p trisomy
(three signals on TP53 FISH). An unbalanced translocation
dic(1,7)(p11;p11), resulting in a trisomy 1q (+1q) and in a mono-
sonsomy 7q, was detected in 3 PI treated ET patients. A fourth
patient carried a +add(1)(p11). A recent study has demonstrat-
ed that dic(1;7) is more frequently observed in secondary
MDS/AML as compared to de novo cases and is significantly relat-
ed to previous treatment with alkylating agents. Usually the time
interval from mutagenic exposure to MDS/AML development is
comprised between 2 and 14 years, similar to that of our 3
Conclude that 17p−, +1q and 7q− are not linked to the natural history of the disease, but, rather they might be induced by the cytotherapeutic treatment given. A short arm deletion of number 17 was detected only in ET patients treated with HU and only on disease transformation. Considering the 11 patients analysed during the follow-up with TP53 FISH, a 17p− was never seen in any of the 8 untreated patients and of the 2 PI treated patients who did not evolve in MDS/AML and it was solely identified at the time of disease evolution in the only HU treated patient. A +1q was detected only in PI treated patients. The fact that in 3 patients it was due to (1;17) and to +add[1](p11) in another one suggests that PI, although acting in a slightly different way, might cause dicentric chromosome formation through a demethylation of the centromeric regions just like alkylating agents.

PO027

CYTOGENETIC ANALYSIS AND FISH WITH A WIDE PANEL OF PROBES IN 150 NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA PATIENTS


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Karyotype is the most important prognostic factor in AML and cytogenetic analysis is still the gold standard for analysing genetic aberrations. Cytogenetics in leukemias has been extensively studied and several recurrent chromosome aberrations have been defined. Major drawbacks to this methodology are that it is expensive, time consuming and affected by a high rate of technical failure. Furthermore 30-50% of pts display a normal karyotype. Molecular techniques such as PCR and FISH detect several specific molecular mutations and are becoming more and more important for genetic screening of leukemias. We analysed 150 consecutive, newly diagnosed AML by classical cytogenetics and by FISH. We used a wide panel of probes: p53, AMI1/ETO; MLL; CBF-β; PML/RAR; c-MYC; RB-1; ET6; cep4; Cep Xy; 20q13; cep7; 7q (q22 and q31); 5q (q31 and q33). The aim of our research was to define better the sensitivity and specificity of FISH compared to cytogenetics and the possible incidence of cryptic aberrations. Karyotype analysis was successful in 70% of cases with 47 pts showing a normal karyotype (39%) and 73 pts (61%) displaying clonal aberrations. FISH analysis compared to cytogenetics showed a specificity and sensitivity of 100% as regards to the following probes: CBF-β (inv16), AM11/ETO; trisomy 8; deletion or monosomy #5 and #7; BCR/ABL. No cryptic genetic aberrations were detected with these probes in pts with a normal karyotype. In the 48 pts whose cytogenetic analysis failed FISH identified 12 cases with genetic aberrations (PM1/RAR=3 pt; MLL=4 pt; monosomy or partial monosomy of #5 and or #7=4 pt and complex karyotype=1 pt). As regards to pts with 11q23 aberrations and successful chromosome analysis in 4 out of 7, FISH was necessary for defining correctly the type of translocation, while in one case with t(12;11) on cytogenetic analysis FISH did not detect the rearrangement. Notably six out 47 pts with a normal karyotype showed cryptic genetic aberrations by FISH analysis: deletion of AMI1=1pts; deletion of ET6= 2 pts; deletion of P53=2 pts and PML/RAR fusion =1 pts. We believe that FISH analysis or multiplex PCR, might be more conveniently used for initial screening of the most common genetic aberrations in AML at diagnosis (PM1/RAR; AMI1/ETO; BCR/ABL; inv16, etc.) including those that might be not detectable with cytogenetics (FLT-3 TID; P53; ETV6 deletion, etc). Cytogenetic analysis should be deserved for all other cases that might harbour rare chromosome anomalies. Nonetheless other tools are needed in order to discover the cryptic genetic anomalies of AML pts with a normal karyotype and lack of known DNA mutations.

PO028

COMBINATION OF LIPOSOMAL DAUNORUBICIN, FLUDARABINE AND CYTARABINE (FLAD) IN PATIENTS WITH POOR RISK ACUTE LEUKEMIA: PRELIMINARY RESULTS

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Introduction. Daunorubicin is one of the most important cytotoxic agents in the treatment of acute leukemia (AL). Its use is usually limited by drug-induced cardiotoxicity depending on the cumulative dose administered. Compared to the unencapsulated anthracycline, liposomal daunorubicin (Daunoxome, DNX, Nexstar) is characterized by a higher tumor cell delivery, improved pharmacokinetic and therapeutic indices, and therefore by a reduced toxicity profile. It has been infact reported that DNX (alone or in association with Ara-C) has a very limited non hematologic toxicity. Patients and Methods. We therefore gave Fludarabine 30 mg/m2 followed after 4 hours by Cytarabine 2 g/m2 (infused in 4 hours) and DNX 100 mg/m2 (infused in 2 hours) × 3 days (FLAD) to investigate the toxicity, safety and efficacy of the regimen in patients with poor risk AL. We have included at now 11 patients: 2 with acute lymphoblastic leukemia (ALL) in relapse (one after allogeneic BMT), and 9 with acute myeloid leukemia (AML). AML patients received FLAD for refractory disease (3), first or second relapse (3, two of whom after autologous bone marrow transplantation) and as first line treatment (2 patients over 60 years of age, one with post-MDS AML). Median age was 53 years (range 13-75). Patients had received a median of 3 prior regimens (range 0-5). Seven patients had intermediate prognosis cytogenetic alterations; poor and good prognosis karyotypes have been detected in 3 and 1 patients, respectively. Median cumulative doses of mitoxantrone was 18 mg/m2 (range 0-120), of idarubicin was 30 mg/m2 (range 0-60). Results. FLAD was well tolerated in most patients. Major infections were observed in 2 patients (sepsis, which responded to specific antibiotic therapy). One patient died 9 days after the completion of therapy due to cerebral hemorrhage. Neutrophil (N > 0.5 × 109/L) and platelet (Plt > 20 × 109/L) recovery required a median of 18 and 18 days from the end of therapy, respectively. As expected, non hematologic toxicity was mild, and in particular no signs of cardiac toxicity have been recorded. Seven responses were achieved (5 CR+2 PR); 2 patients did not respond and 1 patient died before response evaluation. Complete responses were observed in 4 patients with AML (2 treated as front line, 2 treated for relapse) and in 1 ALL patient (in relapse after BMT). Patients treated for refractory AML did not respond. One patient underwent bone marrow transplant (from an unrelated HLA matched sibling) but relapsed at 8 months; the
others are still disease free at 1, 2, 5, and 9 months. Seven patients are still alive, 4 have died (3 for disease progression, 1 for haemorrhage). Conclusions. Our preliminary report shows that FLAD is a feasible and effective treatment in poor prognosis AL, is associated with acceptable tolerability, and does not preclude high dose therapy with autologous or allogeneic rescue.

PO029
SIMULTANEOUS OCCURRENCE OF BCR-ABL AND INV (16) IN A CASE OF M1 ACUTE MYELOID LEUKEMIA

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Cytogenetic analysis performed at the diagnosis is generally recognized as the single most valuable prognostic factor in acute myeloid leukemia (AML). Inv (16) is supposed to be a favourable prognostic factor and the majority of patients with this chromosome abnormality can be cured with conventional chemotherapy, especially when high dose Ara-C (HIDAC) is used. The presence of the Philadelphia chromosome in AML is very rare and characterizes a group of patients with an unfavourable prognosis. The simultaneous occurrence of numeric and structural aberrations in a leukemic blast is a common finding. The association of inv(16) and Ph chromosome has previously been reported in only 6 patients; in all these cases the AML was classified as M4Eo according to FAB recommendations. We describe here the first case to our knowledge of coexistence of CBFβ-MYH11 transcript together with a bcr-abl transcript in the leukemic blasts of an AML M1 case. Case report: A 40 year old female patient was referred to our department in may 2000, because of intermittent abdominal pain, weight loss, fatigue and asthenia. There was no history of leukocytosis and no other signs suggesting a chronic phase of CML or MDS. Routine laboratory findings showed: Hb 12 g/dL, WBC 8 × 10^9/L with 85% blasts and plt 60 × 10^12/L. The bone marrow aspirate contained 90% blasts. The morphological and cytogenetical analysis showed a AML M1 subtype according to FAB classification. On immunophenotype analysis positivity for CD13, CD33, CD34, CD117; 50% of the blasts expressed CD4 antigen. Cytogenetic analysis indicated the simultaneous presence of the Ph chromosome and the pericentric inversion 16 in 18/22 metaphases, whereas in 4/22 metaphases only the inversion 16 was present. RT-PCR analysis confirmed those results. The amplification of the bcr-abl fusion gene revealed a 521 bp fragment which corresponds to the e1-a2 fusion product, indetical to the one normally found in B-ALL. The RT-PCR amplified inv 16 transcript showed a 418 bp fragment. Sequence analysis of this product revealed a new and uncommon breakpoint between the exon 5 of CBFβ and exon 12 of the MYH11 gene with the following sequence: CBFβSCTC ATCCGGGAGAAATGGAGGTCATGAGCTGG AG3 MYH11. The patient received double induction chemotherapy with idarubicin, cytarabine and etoposide (ICE) and achieved hematological and molecular CR in june 2000; she then received 2 monthly courses of consolidation therapy with HIDAC (cycle 1 in August 2000 with Ara-C 6 g/m²; cycle 2 in october 2000 with Ara-C 10 g/m² + lida 10 mg/m²). She was then planned to undergo allogeneic matched unrelated bone marrow transplantation, but in december 2000 while waiting for transplant, she had hematologic relapse and reappearance of bcr-abl and inv(16) transcript on molecular analysis. In january 2001 after a salvage course of chemotherapy with lida 17.5 mg/m² dd 1.8 + Ara-C 3 g/m²/bd dd 2,3,9,10, G-CSF 5 µg/kg/d from d 11 to PMN >15×10^9/L, the patient obtained a second hematological and molecular CR. It is not possible to comment on the impact of these conflicting chromosomal abnormalities on prognosis. Five out of six previously reported cases eventually relapsed. We can only speculate that the good prognosis of AML with inv(16) is probably hampered by the accompanying Ph chromosome.

PO030
UTILITY OF PEROXIDASE ACTIVITY AND NUCLEAR DENSITY ANALYSIS (PANDA) IN DIAGNOSIS AND CLASSIFICATION OF LEUKEMIAS

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Since the first development of automated cytochemistry for leucocyte differential count, a new and efficient approach to leukemia diagnosis and classification was made possible at the time of peripheral blood cell count. This method was progressively improved up to the last version, used by the Bayer ADVIA120 hematology analyzer. It is based on a light assessment of fundamental properties of the main components of cells, such as peroxidase activity (PA) in the Perox channel, which is used to differentiate the main leucocyte types according to the different enzyme content, and nuclear density (ND) in the basophil channel, which is used to count basophils and blast cells and, in association with Perox channel results, flag the presence of abnormal and immature precursors such as blasts, immature granulocytes and necleated red blood cells. Peroxidase activity is a reliable marker of myeloid maturation toward the granulocytic and, to a lesser extent, monocye lineage. The low chromatin density of the nuclei of leukemic blasts permits their identification and differentiation from more mature normal and abnormal leucocytes, as signals that falls close to the origin along the x-axis. This method, indicated as PANDA (Peroxidase Activity and Nuclear Density Analysis), is especially fruitful for the analysis of any type of leukemic populations. When blood samples containing leukemic cells are analyzed by the Bayer hematology analyzers, we obtain detailed informations on their nature, level of maturation or direction of differentiation. The simple observation of PA and ND two-dimensional cytograms can be used to include any single case in a separate and distinct diagnostic category correlated to FAB and WHO classifications of hematological malignancies. This subjective approach to the morphology of leukaemic cell populations on PA and ND cytograms can be made objective using a very simple scoring system, which is the basis of a quick and reliable PANDA classification. This is preliminary to microscope morphology and permits the selection of the most appropriate CDs for immunophenotyping. PA provides information on the myeloid versus lymphoid nature of blasts: lymphoid cells are peroxidase negative (P score = 0), myeloid cells are almost always peroxidase positive with different levels of activity (P score from 1 to 5) according to the level of maturation. ND is very useful in distinguishing between chronic (D score = 0) and acute leukaemias (D score = 1 or 2) on the basis of the loose immature chromatin of blast cell.
nuclei. We have analysed 180 pathological samples at diagnosis (163 with myeloid and lymphoid leukemias and 17 with infectious mononucleosis). Using the PANDA score we have obtained a pre-diagnostic efficiency in 91.1% of the samples. This numerical approach could be easily automated and improved by computer recognition of patterns of distribution of leukemic cell clusters, leading to an expert system for PANDA pre-diagnostic classification of leukemias.

**P0031**
AUTOLOGOUS STEM CELL TRANSPLANTATION FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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High dose chemotherapy followed by autologous stem cell transplantation (ASCT) is increasingly used as consolidation treatment in acute myeloid leukemia (AML) in first or subsequent complete remission (CR). However, most published studies dealing with ASCT exclude patients aged over 60 years, probably due to an anticipated high rate of transplant related mortality (TRM). In this study we analyzed a series of 20 patients with AML in first or second CR aged > 61 years undergoing ASCT. There were 9 males and 11 females with a median age of 64 years (range 61-71). Eighteen patients were in CR1, while 2, including the only patient with acute promyelocytic leukemia in this series, were in CR2. All patients had a performance status 0-1 according to WHO as well as normal cardiac, hepatic, renal and respiratory function. All patients were treated in conventional single rooms. The median interval between CR achievement and ASCT was 4 months (range 2-6). In 18 cases (90%) peripheral blood stem cells (PBSC), collected after consolidation therapy plus G-CSF at a dose of 10 mg/kg, were infused; in 2 patients (10%) bone marrow (BM) was harvested. For patients receiving PBSC, the median number of CD34 positive (CD34+) cells infused was 6.9 × 10^6/kg (range 3-38.8), while 1.2 × 10^6/kg and 2 × 10^6/kg mononuclear cells were given to 2 patients receiving BM. As conditioning regimen BAVC, BuCy2 and L-Bu (idarubicin + busulphan) were used in 11, 5 and 4 patients, respectively. No patient died from transplant related complications and engraftment was achieved in 20/20 (100%) cases. Median number of days to granulocytes > 500/cmm and platelets > 20000/cmm was 13 (range 9-31) and 18 (range 9-19), respectively. One patient failed to achieve platelet count > 20000/cmm, and relapsed at 4 months after ASCT. The median number of platelet and blood units transfused was 4 (range 1-14) and 3 (range 0-14), respectively. G-CSF was administered in 17/20 cases for a median of 12 days (range 6-30). Extrahematologic toxicity included WHO grade III-IV stomatitis in 7/20 (35%) patients and grade IV nausea and vomiting in one case (5%). Furthermore, 13 patients had fever of unknown origin, while 7 a documented infection was diagnosed. Intravenous antibiotics were administered in 19/20 patients (95%) for a median of 10 days (range 3-27). Median number of days of hospitalization was 31 (range 16-60). After a median follow up from ASCT of 7 months, 9 patients are alive in continuous CR. Eleven patients died from AML relapse. Median overall survival (OS) and disease free survival (DFS) from diagnosis were 14 and 11 months, respectively. In conclusion, our data show that ASCT with standard conditioning regimen is feasible in AML patients aged more than 60 years. Toxicity and hematopoietic recovery do not substantially differ from that observed in young-adult AML patients. Results concerning DFS and OS are encouraging, but need a longer follow up to be properly evaluated.
Glutathione S-transferases (GST) are enzymes involved in the detoxification of several environmental mutagens, carcinogens and anti-cancer drugs. GST polymorphisms resulting in decreased enzymatic activity have been associated with several types of solid tumors, including lung, larynx and bladder cancer. The object of our study was the frequency of deletion of two sub-families of GST (GSTM1, and GSTT1, or both) in Italian patients with acute myeloid leukemia (AML). We studied 171 patients (79 females, 92 males, median age: 62 years, range 19-87 years) with AML. Controls were 396 individuals of similar age and sex (177 females, 219 males, median age 65 years, range 19-93 years). All controls had a medical history negative for any type of cancer. A PCR technique which detects homozygous deletions for GSTM1 and GSTT1 was used. Sixty-eight of 171 AML patients (40%) were GSTM1 null, frequencies significantly different from healthy controls (72/396, 18%, OR 3.9, 95% CI 2.2-6.8, p<0.001). The frequency of GSTT1 deletions was also different in the two groups (46/171, 27%, and 48/396, 12%, respectively, OR 2.7, 95% CI 1.7-4.2, p<0.001). Furthermore, the frequency of the double-null genotype, lacking both GSTM1 and GSTT1, was higher in patients with AML (21/171, 12%) than controls (8/396, 2%, OR 6.8, 95% CI 3.1-15.7, p<0.001). The null genotype for GSTM1, GSTT1 or both was particularly frequent in patients over 60 years of age, when compared to controls matched for sex and age (OR: 4.2, 3 and 5 respectively in patients over 60 versus 1.5, 3 and 0.8 in younger patients). No differences were noted when looking at other patients' characteristics, like gender, abnormal karyotype and therapy-related vs. de novo AML. Moreover, GST deletions were negative prognostic factors for the achievement of complete remission following induction chemotherapy and for the probability of overall survival, particularly in elderly patients (median survival 20 versus 9.5 months, in patients with normal GST expression, when compared to GSTM1 and/or GSTT1 deletions, p=0.036). In conclusion, elderly individuals with GSTM1 and or GSTT1 deletions, have an enhanced risk to develop acute leukemia and this phenotype confers resistance to chemotherapy and a shorter survival.

Intensive chemotherapy can induce a complete remission (CR) in 50-60% of patients with poor prognosis AML (with age >60 or previously relapsed) or myelodysplastic syndrome (MDS). However, CR are usually short-lasting (median 6-12 months), only 20-30% reaching a two-year duration (M Beran et al.) Clin Oncol 17:2819; 1999; EH Estey et al. Blood 93:2478; 1999). On the basis of encouraging results obtained in MDS patients ineligible to intensive chemotherapy (D. Ferrero et al. Leuk Res 20:867, 1996; Blood 94 suppl. 1, 1999), we applied to poor prognosis AML and MDS patients, who had obtained a CR by any chemotherapy schedule, a maintenance treatment based on 13-cis retinoic acid (RA) (Roaccutan® 20-40 mg/day) + dihydroxylated vitamin D3 (D3) (Rocaltrol® 1-1.5 micrograms/day) + intermittent low dose 6-thioguanine (6TG) (Thioguanine®) or 6-mercaptopurine (6MP) (Purinethol® 40 - 60 mg/day) for 3 every 5 weeks. In 10 patients the 3 week 6TG treatment was substituted, once every 3-4 months, by a 14 day course of low dose ARA-C (8 mg/m² × 2 /day) + 6MP (40 mg/day). Twenty-one patients (median age 65, 27-73) entered the study. Fourteen had AML, 7 MDS with bone marrow blasts > 10%. In 6 patients the disease was secondary to previous chemotherapy. Four AML patients were in 2nd CR, the other 17 in 1st CR. Remissions had been obtained by different therapies; 16 patients had received standard anti-AML induction (ARA-C at standard or high dose + an anthracycline or mitoxantrone) followed, in 8 patients, by 1-3 consolidation courses; 5 patients had received low dose chemotherapy (6TG ± ARA-C) + RA and D3 in the induction phase too. All patients were not eligible to allogeneic BM transplantation. Maintenance started at a median time of 1 month (range: 0.5 - 3) after CR achievement and has been continued until relapse. Maintenance treatments were well tolerated: mild mucosal dryness due to RA, one case of D3- related symptomatic but reversible hypercalcemia and grade 2-4 thrombocytopenia during ARA-C courses were the only side effects recorded. Median CR duration was 24 months (range -43+), without clear correlation to diagnosis (AML vs MDS) or induction treatment. Six patients (4 AML 2 MDS), all in the group treated with ARA-C containing protocol, are still alive and in continuous CR for 8, 19, 24, 27, 33 and 43 months. In the same group of patients, 3 relapsed and died of AML, 1 died in CR because of 2nd tumor. Our results, although preliminary, suggest that the combination of low- dose chemotherapy (particularly with ARA-C included) + differentiating agents (RA + D3) might be valuable in prolonging remission duration in a proportion of poor- prognosis AML and MDS patients. However, a greater number of patients will be required to confirm these encouraging results.
apy was Fludarabine 30 mg/m² (25 mg/m² in pts. > 70 years) and Ara-C 1 g/m² for 5 days (4 days in pts. > 70 years), in association with Idarubicine 10 mg/m² in 24 hrs. continuous perfusion on days 1, 3, 5. The treatment was followed by administration of glycylsylated G-CSF from day +7 until the end of aplasia (N=1500). A complete remission (CR) was obtained in 15 pts. (62.5%); 5 pts. (20.8%) were resistant and 4 (16.7%) died during therapy (1 cerebral hemorrhage and 1 ARDS) or during the ensuing aplastic phase (1 cardiac arrest, 1 sepsis). The incidence of CR was not different in pts. with RAEB-T and in those with AML (p=0.6). The median duration of hospital stay was 28 days (range 3–73); that of granulocytopenia was 16 days (10–37) and that of thrombocytopenia was 17 days (10–54). A median of 6 days with fever (1–16) was observed. The more relevant infectious complications were: 2 pulmonary aspergillosis (one of which caused the patient’s death while in CR) and infections grade 3/4 according to WHO in 6 more patients. Moreover, two prolonged post-remissional pancytopenias were observed, with positive CMV antigenemia, which resolved after specific antiviral therapy. The pts. achieving CR were treated with a first consolidation course with Fludarabine 30 mg/m² (25 mg/m² in pts. > 70 yrs.), Ara-C 1 g/m² for 2 days and Idarubicine 10 mg/m² on day 2 (given to 14 pts.) and then with a second consolidation course with Ara-C 100 mg/m²/day s.c. and oral 6TG 50 mg/m²/day for 5 days plus oral Idarubicine 15 mg/m² on day 1 (given to 8 pts.). The patients in persisting CR were then given a maintenance therapy with Ara-C (100 mg/m² once a week) and oral 6TG (50 mg/m²/day for 4 days a week) for at least one year or to relapse. The median overall survival (OS) was 8 months, with a minimum follow-up of 1.15 months for censored patients and with an actuarial probability of survival at 26 months of 48%. The median disease-free survival (DFS) was 11.7 months. No statistically significant differences in OS (p=0.7) and DFS (p=0.9) were observed between pts. with RAEB-T and AML. In conclusion a high incidence of CR was observed in the present series of elderly patients. A longer follow-up is needed to prop-

FARNESYLTRANSFERASE INHIBITORS INDUCE CLONOGENIC CELL GROWTH INHIBITION AND APOPTOSIS IN ACUTE MYELOID LEUKEMIA CELLS INDEPENDENTLY BY RAS MUTATION

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The Ras gene transduction pathway is involved in cell prolif-
eration and transformation. Mutationally-activated Ras is the
oncogene found in about 30% of human acute myeloid
leukemias (AML). Ras farnesylation is a requisite for
membrane localization of Ras and is catalyzed by an enzyme termed
farnesyl-protein transferase (FPT-ase). To control cell growth, ras
must move from the cytoplasm to the plasma membrane.
Recently, a variety of inhibitors of FPT-ase, able to block the
growth of Ras-transformed cell lines, have been used as oral
therapeutic agents for the treatment of some types of cancer.
We studied the effects of α-hydroxyfarnesylphosphonic acid and
manumycin-A (FTI), potent and specific inhibitors of FPT-ase,
on cell growth and on clonogenic growth as well as on induc-
tion of apoptosis in the AML cell line KG1a and in primary cells
derived from 50 ALL patients. By allele specific oligonucleotide
polymerase chain reaction, we investigated the presence of
oncogenic N-Ras and K-Ras mutations in primary cells from 40
patients with ALL. By colorimetric MTI and methylcellulose
clonogenic assays, we documented dose dependent FTI-
mediated inhibition of cell and clonogenic growth in KG1a and in
63% and 75% of ALL, respectively. By flow cytometry and DNA
ladder analysis, we documented FTI-mediated apoptosis in 38%
of ALL. Moreover, the addition of Z-DEVD-fmk, an agent that
interferes with caspase 3 activity, partially reverted FTI-induced
inhibition of cell growth and apoptosis of KG1a cells. We docu-
ment oncogenic mutations of N-Ras in 27.5% of ALL; by
contrast, we did not observe K-Ras mutations in any ALL cell
sample. FTI-mediated inhibition of cell growth was observed in
82% and 69% of ALL with and without mutations of N-Ras,
respectively. These findings suggest that FTI may induce growth
inhibition and apoptosis both in Ras-mutated and non-mutat-
ed ALL and may be of therapeutic value in ALL.

Results and outcome. CR was obtained in 3 out of 5 relapsed
patients. Patient #1 relapsed after a matched unrelated trans-
plant and was resistant to FLAG schedule; he obtained CR after
high doses etoposide and then received donor lymphocyte infu-
sion (DLI) to restore donor chimerism. Patient #2 received a MUD
transplant and is in CR at 6 months after transplant; Patient #3

PO036
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A long survival in adult patients with relapsed acute lympho-
blastic leukemia (ALL) can be obtained only with allogeneic
bone marrow transplant (BMT). Second complete remission (CR)
can be achieved in 50% of patients, but is often short and dif-
ficult to maintain until bone marrow transplant. This is partic-
ularly true if the patient has to search for a matched unrelated
donor (MUD). High dose etoposide has been frequently used in
the last years for the treatment of Lymphoma patients; the effi-
cacy of standard doses Etoposide in ALL treatment is well known
and it is often used in consolidation therapy. In our institution
we used 2 g/m² etoposide to treat 5 relapsed ALL patients (4 were
in 2nd relapse) and as consolidation therapy for 3 patients wait-
ning for an unrelated bone marrow transplant.

Characteristics of patients.

<table>
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<tr>
<th>Pts</th>
<th>Age</th>
<th>Phenotype</th>
<th>Response to induction</th>
<th>1RC months</th>
<th>Etoposide treatment</th>
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<td>Resist.</td>
<td>13</td>
<td>2 relapse</td>
<td>CR</td>
</tr>
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<td>2 relapse</td>
<td>CR</td>
<td>23</td>
<td>2 relapse</td>
<td>CR</td>
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<tr>
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<tr>
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<td>CR</td>
<td>34</td>
<td>2 CR</td>
<td>2 relapse</td>
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<tr>
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<td>3*</td>
<td>1 CR</td>
<td>1 CR</td>
<td>CR</td>
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<tr>
<td>8</td>
<td>c-ALL</td>
<td>CR</td>
<td>10</td>
<td>2 CR</td>
<td>2 relapse</td>
<td>CR</td>
</tr>
</tbody>
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sion (DLI) to restore donor chimerism. Patient #2 received a MUD
transplant and is in CR at 6 months after transplant; Patient #3
received BMT from his HLA identical sister, but relapsed 3 months post graft. Patient #6 received an autologous transplant, but relapsed after 6 months. Patients #7 and #8 underwent a MUD transplant: one patient unfortunately relapsed at 9 months, the other is still in CR at 17 months after transplant.

Conclusions. High dose Etoposide is able to obtain or prolong CR in very high risk adult ALL patients waiting for BMT or to treat relapse after transplant with new therapy (DU).

**PO038**

**PGP, MRP, AND LRP EXPRESSION IN 20 PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA AT ONSET AND AFTER RELAPSE**


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P-glycoprotein (PGP) expression is one of the most important mechanisms involved in the Multidrug Resistance (MDR) phenomenon. More recently, the Multidrug resistance related protein (MRP) and the Lung resistance protein (LRP) were related to the atypical MDR. In this study we evaluated PGP, MRP and LRP expression by flow cytometry in 20 patients studied at onset and after relapse using the monoclonal antibodies MRK16 (anti-PGP), MRPm6 (anti-MRP) and LRP56 (anti-LRP). At onset, 10/20 (50%) cases overexpressed PGP (PGP+) (MFI=7.1±5.2), 2/20 (10%) MRP (MRP+) (MFI=1.7±0.9) and 2/20 (10%) LRP (LRP+) (MFI=3.5±2.9). The MDR phenotype of these 20 patients was studied again after the first relapse: 6/10 (60%) were PGP+ (MFI=7.2±3.0), 4/10 (40%) MRP+ (MFI=1.8±1.3) and 6/20 (30%) LRP+ (MFI=4.0±3.3). Ten of these patients were studied in second relapse: 6/10 (60%) were PGP+ (MFI=7.2±3.0), 4/10 (40%) MRP+ (MFI=2.8±2.0) and 6/10 (60%) LRP+ (MFI=7.1±3.3). In conclusion, our data show that PGP is frequently overexpressed at onset while MRP and LRP are more rarely overexpressed. In relapse we observed an increase of PGP, MRP and LRP overexpression rates and of the fluorescence intensity, particularly for LRP. These data suggest that in adult acute lymphoblastic leukemia there is a rapid induction of MDR related proteins, already by first relapse, and this must be taken into consideration for the choice of chemotherapy.

**PO039**

**ANALYSIS OF LINEAGE INVOLVEMENT IN PHILADELPHIA CHROMOSOME POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA**

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A review of the literature reveals that the issue of which level of hemopoiesis the de novo Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph-positive ALL) arises at is unresolved. Actually, while ALL was believed to be restricted to the lymphoid lineage, the existence of stem cell ALL based on the involvement of myeloid elements in the leukemic process has been postulated. Alternatively, it has been reasoned that multilineage involvement represents chronic myeloid leukemia in lymphoid blast crisis (CM-LBCC) because the 2 entities are sometimes not easily distinguished. To gain further insight into this controversial problem, we examined the expression of p190 or p210-type bcr-abl mRNA in single hematopoietic colonies (CFU-Mix, BFU-E, CFU-GM) obtained from either BM or PB of 7 Ph+ ALL patients with no history of chronic myeloproliferative disorder, using RT-PCR. At diagnosis a mean of 38 colonies (range from 20 to 70) was analyzed for each patient and the bcr-abl transcript was demonstrated in 17 to 35% of these in 5 out of 7 patients (2 with M-bcr and 3 with m-bcr rearrangement). We also evaluated the involvement of the myeloid compartment in 3 of 5 positive patients, during different clinical stages when they were in complete remission: 2 patients (with m-bcr rearrangement) presented a decrease of the percentage of positive colonies (range from 4 to 17%), while one patient (with M-bcr rearrangement) a considerable increase (range from 34 to 47%) proving to be a case of CM-LBC reverted to chronic phase. In conclusion, our data demonstrated that: (i) de novo Ph+ ALL, unlike Ph−, can originate in pluripotent progenitor cells; (ii) the analysis of the lineage involvement during follow up could be useful in the discrimination between CM-LBC and de novo ALL with similar clinical and biological features at diagnosis. Taken together these results could be important to understand the pathogenetic mechanisms of disease and to determine the leukemic target cells in ALL, for the development of selectively antileukemic therapeutic or purging strategies.

**PO040**

**BONE MARROW-, BUT NOT TYMUS-, DERIVED STROMAL CELLS INHIBIT SPONTANEOUS APOPTOSIS AND INDUCE PROLIFERATION IN BLASTS OF T-LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA**

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When blasts of T-lineage ALL are placed in a culture medium, the majority of cells die by apoptosis within 72 hours. In this study we investigated the possible control of BM- and thymic-stromal cells on T-ALL cell survival. To this aim we performed co-cultures between blasts obtained from 5 adult T-ALL patients and confluent layers of normal human BM or thymic epithelial (TE) cells. The spontaneous apoptosis observed in cultured blasts was completely abolished when they were co-cultured with BM stromal cells. In contrast, the interaction with TE cells had no effect on cell survival. To extend these findings to a non-pathological setting, we co-cultured normal, unfractionated thymocytes with BM or thymic stromal cell layers. The thymocyte spontaneous apoptosis was completely inhibited by the interaction with BM-, but not thymic-, stromal cells. In addition, rescued ALL blasts, but not normal thymocytes, were induced to proliferate by the interaction with BM cells. When BM stroma was replaced with established cell lines used as controls, no protective or proliferative effects were observed. Both ALL protection
and proliferation were dependent upon cell-cell adhesion, as BM-derived supernatant was unable to protect or induce proliferation in immature T cells. These results point out the role of BM stroma in the regulation of T blast survival. Furthermore, they highlight the preserved ability of normal and neoplastic T cell precursors to respond to survival stimuli from BM microenvironment. This mechanism, strengthened by the proliferative potential of T blasts, could play a role in the blast spreading to BM during pathogenesis of T-ALL.

PO041
HIGH DOSE IDARUBICIN PLUS HIDAC AND AMIFOSTINE IN INDUCTION CHEMOTHERAPY IN ELDERLY PATIENTS WITH ACUTE NON-LYMPHOCYTIC LEUKEMIA

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Elderly patients with ANLL have a poor prognosis: the Remission Rate After Induction CHT is lower than younger patients and even after the achievement of CR, the intensive consolidation with autotransplantation is feasible in very few cases. In this subset of patients, the main causes of treatment failure include the poor tolerance of aggressive chemotherapy and the intrinsic disease resistance. We used Amifostine as cytoprotective agent in addition to a well known and successful chemotherapy regimen, containing high dose (HD) Idarubicine and HD ARA-C, the so-called Memorial, currently used in younger patients with ALL. We treated 20 elderly patients (18 ANLL, one Chloroma and one blast crisis of MF) (median age: 67, range: 56-78). Amifostine was administered on the 3rd day at dosage of 740 mg/m² IV, followed by Idarubicin at 40 mg/m², ARA-C was infused I.V. at 3 g/m² from day 1 to day 5. Patients aged more than 70 yrs received 30% reduced schedule. Karyotype was normal in 5 pts, one patient presented the trisomia 8, unfavorable karyotype in 9 pts, un evaluable in 5 pts. We observed one toxic death due to infection. Thirteen out of the 19 evaluable responses were complete, the blast crisis of MF achieved the chronic phase. The median time to reach a neutrophil count > 500/µL, and > 1500/µL was 17 (range: 9-29) and 22 days (range: 15-31) respectively. Platelet counts >20,000/µL >50,000/µL, >100,000/µL were achieved in median at d +20 (range: 14-38), d +24 (range: 16-52), d +25 (range:17-98) respectively. All pts. experienced a grade III infectious episodes which promptly responded to antibiotic IV treatment. The other grade III toxic events included diarrhea and hepatic dysfunction in 4 and 2 pts respectively. Grade III-IV oral mucositis was never observed. In all pts. the duration of the extrahematological toxic events was very short and did not prolong the hospital stay (median: 31 days). In 9 out of the 13 pts. we were able to collect a median of 7.1 x 10^6 CD34+/kg (range 3.5-14) after a consolidation course with FLAG+Daunoxome. Seven of these 11 pts were autotransplanted with a conditioning regimen including Busulfan 9 mg/m², Melphalan 120 mg/m² in combination with Amifostine and Daunoxome 160 mg/m². We observed 2 toxic deaths due to ischemic colitis and to acute myocardial infarction. Our preliminary experience suggests that HD-IDa + HD-ARA-C, when associated with Amifostine, is a very effective and well tolerated induction regimen, also in elderly patients with ANLL; this treatment does not impair the collection of peripheral stem cells, allowing to perform also intensive consolidation in a high percentage of patients. A longer follow-up and a larger cohort of patients are needed in order to evaluate the duration of response and its possible correlation to karyotype status.

PO042
QUANTIFICATION OF ANGIGENESIS IN ACUTE MYELOID LEUKEMIA: A MULTIPARAMETRIC COMPUTERIZED IMAGE ANALYSIS MODEL

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There is considerable experimental evidence to indicate that tumor growth is dependent on angiogenesis and its key role in solid tumor growth is well established. In contrast, few data are available for hematologic malignancies, especially concerning bone marrow neo-vascularization in acute leukemia. We evaluated 13 acute myeloid leukemia (AML) bone marrow biopsies. We used MoAb VEGF, to detect the production of this factor by blasts cells, and MoAb vWF to detect endothelial cell. Paraffin biopsies were treated with immunohistochemistry using the APAAP method. Quantification analysis of stained sections was performed by multiparametric computerized image analysis. In all cases (13/13) of AML we detected VEGF positivity with range 42-85% (mean value 66% of bone marrow cellularity). In contrast, in 3 negative biopsies we detect VEGF minimum expression (≤ 5% of bone marrow cellularity). This difference was found significantly in statistical analysis (p<0.0001). In all cases, to study neo-vascularization, we valued, by multiparametric image analysis, these parameters: mean area, per cent mean area, density. In positive biopsy our results were: per cent area 8.7% (range 5.3-14.5%), density 2981.76 (range 436.81-5316.01), in negative biopsy we observed: per cent area 0.47% (range 0.41-0.52), density 8.53 (range 4.13-13.91). These differences were found significantly in statistical analysis (p<0.0001). In each cases of AML we observed high grade of vascularization and an equivalent high VEGF expression (p=0.001 correlation test). In conclusion, this study confirm: vascularization has risen in AML bone marrow, neo-vascularization, in this hematological malignance, could be an important marker to monitor leukemia response during treatment and in predicting leukemia relapse, multiparametric computerized image analysis is more objective method and the results are repeatable.

PO043
DOWNMODULATION OF ERK KINASE ACTIVITY INHIBIT THE PROLIFERATION OF ACUTE MYELOID LEUKEMIA BLASTS

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Extracellular signal-regulated kinase (ERK) is thought to play an important role in integrating and transmitting transmembrane signals required for cellular growth and differentiation.
Constitutive activation of ERK has been observed in some human malignancies, including acute leukemia. In this in vitro study, we treated with a selective MEK1 inhibitor, PD98059 (New England Biolabs, Beverly, MA, USA), 14 cases of acute myelogenous leukemia (AML) with high MEK1/ERK 1/2 activity and 4 cases with undetectable or very low activity of these kinases. After three hours of incubation of AML blasts with high ERK activity with PD 98059 at concentrations of 20 µM and 40 µM the MEK1/ERK1/2 inhibition was evident in some samples (9 of 14), the MEK1/ERK1/2 down modulation at these concentrations was persistent for 6, 12, 18 and 24; after 24 hours of treatment, we observed a strong downmodulation of ERK1 and ERK2 dual phosphorylation and a strong decrease of the levels of ERK1/2 activity in all the samples with high MEK/ERK activity. The mean ± SD of decrease of the levels of ERK 1/2 activity, after 24 hours of treatment at concentrations of 20 µM and 40 µM, was 55.8 ± 13.9 and 82.5 ± 12.6 respectively. The treatment downmodulated MEK1 phosphorylation also. Tritiated thymidine uptake assay showed significant decrease of cell proliferation in comparison with untreated controls. By incubating 36 hours, at concentrations of 20 µM and 40 µM, we detected 17.2 ± 12.25 and 38.5 ± 10.5 inhibition. By incubating 60 hours, we detected 35.92 ± 13.19 and 50.54 ± 17.71 inhibition. In contrast, the proliferation of the blast cells that did express low or undetectable levels of ERK activity was not inhibited. We made other experiments by starving for 24 hours the leukemic cells, and then by adding for other 24 hours the PD98059. After this time of exposure, we stimulated the leukemic cells with FCS, GM-CSF or FCS + GM-CSF for 10 minutes. Even at the concentration of 20 µM, PD98059 strongly inhibited the capability of growth factors to activate ERK in comparison with untreated controls and significantly downmodulated leukemic blasts proliferation. These experiments demonstrated that the inhibition of MEK1 by PD98059 is selectively capable to block ERK activation and to downmodulate the proliferation of primary AML blasts with high levels of ERK activation. Interestingly, ERK downmodulation by PD 98059 induced, by Caspase-3-mediated cleavage of poly (ADP-ribose) polymerase (PARP), the appearance of p89PARP, an early sign of apoptosis, followed then by internucleosomal DNA fragmentation. We observed that the cleaved p89PARP appeared after 18 hours of PD98059 (20 and 40 µM) in 8 of 14 cases whereas after 24 hours of treatment cleaved p89PARP was present with different levels of expression in all the 14 cases with active MEK1/ERK1/2 whereas in the negative MEK1/ERK1/2 cases we did not observe any cleaved PARP fragments even after 24 and 48 hours incubation. Clonogenic assays demonstrated that the treatment of normal hematopoietic progenitors with PD 98059 (20 µM and 40 µM) did not induce any hematopoietic cytotoxic effects in CFU-Mix, BFU-E and CFU-GM. Our results prompt us to perform in vivo studies to control the proliferation of AML blasts by MEK1 inhibitors.

PO045
CD34+ CD2+ ACUTE PROMYELOCYTIC LEUKEMIA (APL): IS THIS A DISTINCT SUBSET?

Compared to other acute myeloid leukemias, APL displays a distinctive immunophenotypical profile including CD33, CD34, and CD9 antigen expression and negative staining for HLA-DR. We investigated CD34 and CD2 expression on APL blast cells to verify the possibility of distinguishing a subset with some biological characteristics. We analyzed CD34 and CD2 expression in 114 newly diagnosed APL patients. Median age was 42 yrs (range 5–88 yrs). One hundred and three (90%) cases were classified as M3 and 11 (10%) as M3 variant (M3v). The parameters considered were WBC and PLT counts, hemoglobin (Hgb) levels, percentage of peripheral blood blasts, CD15, CD56 and HLA-DR expression, and the PML/RARα isoform, to assess their relationship with CD34 and CD2. Cut-off values for defining antigen positivity were >10% for CD34, and >20% for the other markers. Two patient groups could be identified: CD34+ CD2+ (20 cases), CD34- CD2- (66 cases); the remaining 28 patients could not be considered because they had heterogeneous expres-
sion of CD34 and CD2. The M3v cases were 5; these cases were associated with CD34+ CD2- (p = 0.004) group. The median WBC count was 11.8×10^9/L and 1.8×10^9/L for the CD34+ CD2+ and CD34- CD2- groups, respectively (p = 0.004). The median PLT count was lower in CD34+ CD2+ than in CD34- CD2- cases (19.5 ×10^9/L vs 27.5×10^9/L, p = 0.01). The median percentage of peripheral blood blast cells was higher in CD34+ CD2+ than CD34- CD2- patients (88% vs 18%, p = 0.0002). There was no significant difference between the two groups in terms of age, sex, CD15, CD56 and HLA-DR antigen expression. The BCR3 rearrangement type was associated with the CD34+ CD2- group (p=0.008). When M3v cases were excluded, the CD34+ CD2+ and CD34- CD2- groups still had statistically different values for WBC, PLT count and percentage of peripheral blood blasts. Moreover, CD15 expression was associated with the CD34- CD2- group (p = 0.03). Our results show that in APL the CD34+ CD2- phenotype is associated with higher WBC and lower PLT counts and a higher percentage of peripheral blood blasts. These data suggest that immunophenotypical analysis could distinguish an APL subset with different biological characteristics. Further studies in homogeneously treated APL patients are needed to assess the prognostic impact of this immunophenotypical pattern.

PO046 IMMUNOPHENOTYPIC CHARACTERIZATION OF BLAST CELLS IN TYPICAL ACUTE PROMYELOCYTIC LEUKEMIA AND ITS VARIANT: A SINGLE CENTER REPORT

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It is well known that t(15;17) acute promyelocytic leukemia (APL-M3) is characterized by a high response to therapy with all-trans retinoic acid, thus early diagnosis is essential and it depends primarily on morphological recognition of leukemia promyelocytes on blood and/or bone marrow smears since cytogenetic or molecular data can rarely be available in real time. In order to assess the immunological pattern of blast cells in classical and variant APL (M3v) and the diagnostic value of immunophenotyping in APL, we evaluated by three colour flow cytometry the leukaemic immunophenotype in 9 cytogenetically and molecularly confirmed APLs. Seven of 9 were diagnosed as typical hypergranular APL, while 2/9 were M3v, because of the presence of hypogranular bilobed cells. We used a large panel of monoclonal antibodies: CD3, CD19, CD33, CD34, CD2, CD13, CD15, CD117, CD9, CD16, CD11b, CD56, HLA-DR, cyMPO and CD45. Data acquisition and analysis were performed using the CellQuest software and a FACSCalibur flow cytometer (Becton Dickinson). Events were acquired with Side Scatter parameter (SSC) in logarithmic scale and dot plot representation was SSC/CD45 PerCP to better isolate the blast population. The positivity for a given monoclonal antibody was attributed when it was expressed by at least 30% of blast population. We observed in all cases a well-defined SSC/CD45 distribution pattern, with blasts showing very high SSC and low expression of CD45. This finding was observed in all studied cases of APL, irrespective of the M3 or M3v morphology; in contrast, we rarely found it in cases of AML other than APL. Immunological phenotype of classical hypergranular M3 blasts invariably resulted the following: CD33+CD13+CD117+cyMPO+CD9+CD16+CD11b-HLA/DR-CD34-. One case resulted also positive for CD56, a neural adhesion molecule associated with adverse prognosis according to some reports. Microgranular variant immunophenotype did not differ dramatically, except for the aberrant expression of CD2 in both two cases of M3v, thus confirming similar data from literature. In contrast with other AML subtypes, where poor correlation is generally found between immunophenotype and morphology, we found a quite typical immunophenotype in APL, especially in the classical hypergranular form and a peculiar light scatter pattern which could be useful for the diagnosis of M3v, which is potentially difficult to recognize because of the morphology of blasts often resembling monocytes. Immunophenotyping in APL may thus represent an effective, rapid and reproducible measurement system which could improve diagnosis and be useful when cytogenetic or molecular data are not yet available and morphology is not clear.

PO047 BONE MARROW NECROSIS PRECEDING ACUTE MONOBlastic LEUKEMIA: A CASE REPORT

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Bone marrow necrosis (BMN) is a rare pathological finding described as necrosis of both the parenchimal and stromal components in large areas of hematopoietic bone marrow. Little is known about this entity though it was first described more than 50 years ago. In the majority of cases it represents an autopsy finding, a malignancy usually being the underlying disease. We describe here the case of a 71-year old woman with a picture of BMN preceding an overt acute monoblastic leukemia. The patient was admitted to the Hematology Unit in January 2001 with a history of diabetes mellitus and the recent emergence of asthenia, pallor, abdominal pain and melena. On admission her hemoglobin was 6.5 g/dL, RBC 2.12×10^12/L, hematocrit 18.0%, MCV 76 fL, WBC 1.3×10^9/L, platelets 18.0×10^9/L. Differential cell counting was as follows: (neutrophils 45%, lymphocytes 45%, monocytes 10%). Schistocytes were not detected on the peripheral blood smear. Serum LDH was markedly elevated (1545 U/l). Antiglobulin test was negative. Bone marrow aspiration on multiple sites only revealed an amorphous eosinophilic material surrounding cellular ghosts with indistinct margins. Cell nuclei showed pyknosis and karyolysis. Her fibrinogen was The patient was transfused with packed red cells and platelets, and received intravenous somatostatin. Bone marrow trephine biopsy confirmed the diagnosis of BMN. CT total body scan did not reveal a malignancy. The following week the patients showed a rising WBC and platelet count, WBC 2.0×10^9/L (neutrophils 1.3×10^9/L, lymphocytes 0.3×10^9/L, monocytes 0.3×10^9/L), platelets 61.0×10^9/L and a fall of LDH to 494 U/l. She was discharged and followed on an outpatient basis till March 2001, when she developed widespread, papuloid skin lesions. Her hemoglobin was 7.1g/dL, RBC 2.48×10^12/L, hematocrit 19.0%, WBC 22.0×10^9/L (blasts 80%), platelets 30.0×10^9/L. The LDH level was 2150U/L. Her bone marrow aspiration smears now showed a picture of diffuse infiltrat-
tion by undifferentiated blasts that proved of monoblastic origin on cytochemistry and flow-cytometry. The patient then received a chemotherapy course with FLAG (fludarabine at dose 30 mg/m²/d, Ara-C 2 g/m²/d for three days and G-CSF), but died of bronchopneumonia ten days later. From the clinical point of view, BM N seems to be extremely rare as evidenced by few reports in which BM N was diagnosed during life in the literature so far. The percentage of BM N associated with acute myeloid leukemia is 13%; generally BM N follows acute leukemia and the prognosis is extremely poor. Our case report confirms poor prognosis but is unusual for BM N preceding AML. The patients who recover from BM N need very frequent observation.

PO048
THE HYBRID GENE bcr/abl IS A NEGATIVE PROGNOSTIC FACTOR EVEN IN ACUTE MYELOID LEUKEMIA

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The bcr/abl hybrid genes are typically associated with leukemic disorders: p210 is the hallmark of CML, p190 is often found in ALL, p230 denotes CMML. The presence of Ph+ AML are reported in the literature. We have looked for Ph+ and/or bcr/abl rearranged cases in two recent GIMEMA trials for AML. In the AML-10 study, of 1226 patients registered, cytogenetic and/or molecular data were available in 548 cases (44%), and 10 patients (1.8%) could be defined Ph+ AML. In the AML-99P pilot trial, of 406 patients enrolled, 306 (75%) were tested and 5 (1.6%) were Ph+ AML. Pooling these data, we have collected 15 Ph+ AML patients (which is the largest series reported in this setting), aged 20-58 years (median 47.5). We have compared disease characteristics and outcome with those of the whole cohorts and of the Ph- patients. Preliminary findings of this analysis show that the percentage of Ph+ patients tends to increase with age (5 patients were less than 40 y, 10 more than 40); the majority of cases had a percentage of Ph+ patients (which is the largest series reported in this setting), aged 20-58 years (median 47.5). We have compared disease characteristics and outcome with those of the whole cohorts and of the Ph- patients. Preliminary findings of this analysis show that the percentage of Ph+ patients tends to increase with age (5 patients were less than 40 y, 10 more than 40); the majority of cases had a

PO049
SCORING SYSTEMS AND PROGNOSIS IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA CO-EXPRESSING MYELOID ANTIGEN

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We analysed clinical and immunological characteristics of 140 adult acute lymphoblastic leukemia (ALL) cases diagnosed in the last 12 years. Immunophenotyping was performed to classify ALL as B or T lineage and to identify ALL with myeloid antigen co-expression. The scoring systems proposed by Catovsky and EGIL Group were applied to qualify cases with score 0 (group 1), between 0.5 and 1.5 (group 2) and 2 (group 3). The study does not include trues biphenotypic leukemias (score > 2). Of the 140 cases, 37 (26%) and 23 (17%) belonged respectively to the group 2 and 3. No correlation with L1/L2 morphology and B/T lineage was found. Median age (52 yrs. vs 32 and 33; p = 0.01), Ph+ incidence (39% vs 19% and 16%; p = 0.05) and WBC count (54 vs 34 and 33×10⁹/L; p = 0.04) were significantly higher in group 3 with respect to groups 2 and 1. Co-expression of CD34, CD117, CD133, CD90, CD25 and CD45RA was tried: the group 3 expressed more frequently CD25 (31% vs 12% and 7%; p = 0.05) and CD45RA (93% vs 61% and 51%; p = 0.02): incidence of CD34 expression was over 70% in each group and of CD117, CD133 and CD90 was desultory and without a relationship with the score. Cases receiving standard ALL therapy were 129 (group 1: 76 - group 2: 34 - group 3: 19). In the group 3 was observed the lowest CR rate (68% vs 85% and 83%; p NS). Surprisingly, DFS had a more positive trend in this group with respect to group 2 and 1: the median DFS was 86, 18 and 30 months and the estimated DFS was 40%, 8% and 24% in the groups 3, 2 and 1, respectively (p = 0.027). The OS was not significantly different among the groups. Multivariate analysis showed an irrelevant influence on DFS and OS of My-Ag co-expression. In this series only Ph+ and WBC count had an independent impact on the outcome. In our experience the scoring system in adult ALL doesn’t seem to identify a group with different risk. However, the group with score 2 was frequently associated with higher age, WBC count, Ph+ incidence, resulting more refractory to induction therapy. But, just in this group the sensitive cases showed a greater capacity to persist in the condition of CR.
performed 2 presented chromosome 3 abnormalities. At diagnosis cutaneous infiltration. Twelve pts (52%) reached a CR, 5 pts (22%) died in induction and 6 pts (26%) were unresponsive. The median duration of CR was 33 weeks (range 10-441). Four patients died in CR due to chemotherapy-related complications: in 3 pts during consolidation course and in 1 patient during aBMT. Seven patients underwent transplantation procedures (1 BMT, 4 aBMT, 2 aPBSCT). Comparing the CR rate between AML patients and the other cases of AML, no difference was observed (12/23 (57%) vs 1918/3329 (57%), OR 1, 0.39-2.57, p=ns). These results were mirrored for different age groups. The median survival was 40 weeks (2-444). At present, after a follow-up of a minimum of 1.9 years, only 2 pts are alive in CR, all the other pts died. A 5-yr Kaplan-Meier curve showed 16% of DFS and an actuarial overall survival of 8.7%. Conclusions. AMeL is a rare form of AML. The CR duration and the overall survival in this group of pts are very poor. Furthermore, a high number of deaths in CR was observed. Considering the low incidence of this kind of leukemia, a specific therapeutic approach could not add significant data, due to the scarcity of pts available in a brief period. However new strategies could be considered with a view to reducing the death rate in CR.

**PO0051**
**ACUTE MEGAKARYOBLASTIC LEUKEMIA: EXPERIENCE OF GIMEMA TRIALS**


**Objectives.** To evaluate the incidence, characteristics, treatment and outcome of acute megakaryoblastic leukemia (AMeL) in patients (pts) enrolled in GIMEMA trials. Patients: Between 1982 and 1999, 3352 new consecutive cases of AML aged over 12 yrs were admitted to GIMEMA trials (LANL 8201, LANL 8202, AML 8A and 8B, AM10, LANL 0491 and 0594). Of them, 23 were AMeL. Results. The incidence of AMeL among AML enrolled in GIMEMA trials was 0.7% (23/3352). The median age was 51 yrs (15-76). M/F ratio was 14/9. W.H.O. performance status was: 0 in 6 pts, 1 in 10 pts, 2 in 5 pts, 3 in 2 pts. Morphological diagnosis was confirmed in 70% of cases by the reference board. Immunophenotype was determined in 14 pts (61%) and in all cases immunologic features of megakaryoblasts were expressed (CD41+, CD42+, CD61+, or FVIIIag+). Out of 7 cytogenetic studies performed 2 presented chromosome 3 abnormalities. At diagnosis the median WBC count was 6.3x10⁹/L, platelet count 64x10⁹/L, and Hgb level 7 g/dL. LDH had a median value 2.5 higher than normal value (600 IU/mL, range 285-4000). Two patients (9%) presented at diagnosis cutaneous infiltration. Twelve pts (52%) reached a CR, 5 pts (22%) died in induction and 6 pts (26%) were unresponsive. The median duration of CR was 33 weeks (range 10-441). Four patients died in CR due to chemotherapy-related complications: in 3 pts during consolidation course and in 1 patient during aBMT. Seven patients underwent transplantation procedures (1 BMT, 4 aBMT, 2 aPBSCT). Comparing the CR rate between AML patients and the other cases of AML, no difference was observed (12/23 (57%) vs 1918/3329 (57%), OR 1, 0.39-2.57, p=ns). These results were mirrored for different age groups. The median survival was 40 weeks (2-444). At present, after a follow-up of a minimum of 1.9 years, only 2 pts are alive in CR, all the other pts died. A 5-yr Kaplan-Meier curve showed 16% of DFS and an actuarial overall survival of 8.7%. Conclusions. AMeL is a rare form of AML. The CR duration and the overall survival in this group of pts are very poor. Furthermore, a high number of deaths in CR was observed. Considering the low incidence of this kind of leukemia, a specific therapeutic approach could not add significant data, due to the scarcity of pts available in a brief period. However new strategies could be considered with a view to reducing the death rate in CR.

**Introduction.** Reported CR rate in elderly AML is approximately 50% in most studies with ara-C/daunorubicine regimens, or higher in more recent FLAG protocols. Early deaths, concomitant diseases, biological factors contribute to the poor outcome. In HR-MDS (RAEB-T, mixed MDS/MPD, high IPSS score MDS) AMeL regimens are increasingly used. In some trials amifostine has improved cell counts and transfusion requirement. Little data exists on its association with conventional dose ara-C/mitoxantrone regimens. Patients and Methods. 12 AML (no M3) and 6 HR-MDS patients (3 RAEB; 3 RAEB with IPSS > 2; 2 MDS(MPD) were treated with the MP (standard dose ara-C and mitoxantrone; Yin et al. Br J Haematol 1991, 79, 415) and MP+AMF respectively (see Table for details). Results. 10 (83%) and 4 (50%) AM and HR-MDS patients respectively achieved hematological CR and cytogenetic remission (KR) with 1 (10 patients) and 2 (5 patients) induction courses; there were no infectious or haemorrhagic deaths; one induction death was due to disease progression. CR and KR were higher in AML than in HR-MDS. Infectious and bleeding episodes and median hospitalisation time (5 weeks) for each chemotherapy course were similar to adult AML, but CR duration and long term survival remain short. Of the 12 AM patients, only 2 (16%) survive 20 and 32 months from diagnosis; 1 in CCR received autologous stem cell transplantation; 1 relapsed 12 months from CR and has achieved a 2nd CR with G-CSF. All other AML patients died of relapse or resistant disease 0-72 weeks from CR (median 38). Of the 8 HR-MDS patients, 2...
(25%, 1 with AREB and 1 with MDS/MPD) are alive 19 and 26 months from diagnosis but relapsed 14 and 17 months from achievement of CR. Conclusions: MP has high CR and KR rates in elderly AML patients; little toxicity, average hospitalisation times, allows good quality of life: responding patients spend long spells at home, have few hospital visits, fewer or no medications or transfusions. However, most patients relapse and die of leukemia within a year and are seldom salvageable because of their age. More effective non-toxic post-induction therapy is needed for this age group. A synergy MP/AMF in HR-MDS is not immediately apparent in our small experience and should be evaluated in larger studies, which are hampered by costs. The new WHO classification equates RAEBT with de novo AML; some cases may be true early diagnosed AML, most may be MDS in progression and may require different treatment programs.

<table>
<thead>
<tr>
<th>Patients</th>
<th>M/F</th>
<th>AML/MDS</th>
<th>Median age (range)</th>
<th>Abnormal karyotype</th>
</tr>
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<tbody>
<tr>
<td>9/12</td>
<td>12/8</td>
<td>70 (60-82)</td>
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- **10/4 (83/4)**
- **11/8 (92/100)**
- **2/2 (1/7)**

*AML/MDS (%)

**P0053**

**POST-REMISSIONAL MAINTENANCE THERAPY IN ELDERLY PATIENTS WITH AML**

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With the expansion of the elderly population, there is an increased incidence in acute myeloid leukemia in this age group. In patients >70 years of age treated with aggressive chemotherapy, the percentage of complete remissions is not more than 40% and mean survival is less than 12 months. With non-aggressive induction therapy or only supportive treatment, mean survival is only about 4 months. Past studies in patients > 60 years have shown that maintenance therapy prolongs DFS but does not modify overall survival. We evaluated post-remissional therapy on DFS and on OS and its tolerability in a group of patients > 70 years or younger but in particularly compromised condition. Fourteen patients (8 males, 6 females) of median age 72 years (range 60-84) with AML in complete remission were evaluated. The staminal immunophenotype was present in 8/4 patients; 11 patients had concomitant diseases, 5 cardiovascular and 6 endocrine. Twelve were in first remission and 2 in second remission. Seven patients had undergone chemotherapy with ARA-C + Thioguanine, 4 with DAT and 3 with intensive regimens. All patients began treatment with ARA-C 100 mg/m² bid + Thioguanine 100 mg/m² bid for 5 days a month from complete remission up to relapse. Seventy-five cycles were completed and well-tolerated in 14 patients. Hematologic toxicity with severe leukopenia (<500) and thrombocytopenia (<10,000) occurred in only one patient and lasted 4 days. A total of 23 transfusions were required and 2 platelet infusions were needed in 5 patients; 9 patients did not require supportive therapy. Only 3 mild infective episodes occurred; 2 of which resulted in hospitalization. Therapeutic cycles did not require prolonged hospitalizations beyond the expected time span for administration: 62.5% of patients is alive at 43.4 months with a median survival of 19.3 months (range 4.4-43.4). The duration of complete remission was 15.2 months. Median survival has not been reached. Though the number of cases is small, there seems to be an advantage in post-remission therapy on DFS in elderly patients with AML.

**P0054**

**LIPOSOMAL DAUNORUBICIN (DAUNOXOME) FOR TREATMENT OF POOR-RISK ACUTE LEUKEMIA**

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Host-tissue toxicity limits use of anthracyclines in elderly, sick patients and in heavily pretreated or relapsed patients who are eligible for bone marrow transplantation (BMT). Since the liposomal preparation of Daunorubicin (DNR) (DaunoXome, or DNX) is less toxic than conventional DNR, we tested DNX, 80 to 120 mg/m² daily for 3 days combined with high dose arabinosyl cytosine (2000 mg/m² daily for 5 days) in 42 adult poor-risk acute leukemia patients. Among 31 acute non-lymphocytic leukemia (ANLL) patients there were 16 (51%) complete remissions (CR), 5 deaths during induction and 10 failures; survival and disease-free survival (DFS) were 25% and 23% at medians of 8 months (1-24) and 7 months (1-13) respectively. Among 11 acute lymphocytic leukemia (ALL) patients in first or subsequent relapse during chemotherapy there were 10 (91%) CRs and only one failure; survival and DFS were 38% and 23% at medians of 7 (4-15) and 6 months (2-14) respectively. CRs were seen equally among multidrug resistant patients, and no relationship with cytogenetics or other factors was detected. Hemopoietic toxicity was substantial, with 3 cases of fatal infection and 2 of fatal hemorrhage. Non-hemopoietic toxicity was negligible. The low frequency (2%) of Gram-negative bacteremia probably depended on absence of intestinal toxicity thanks to reduced disruption of the GI mucosal barrier. We conclude that DNX can substitute free DNR in acute leukemia patients; the high CR rate observed in ALL requires confirmation.
Hodgkin’s Disease

PO055
INCREASED INCIDENCE IN HIV-ASSOCIATED HODGKIN DISEASE AFTER THE INTRODUCTION OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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The recent introduction of HAART has changed the natural history of HIV infection and could have also influenced the development and outcome of HIV-related lymphomas. While the incidence of primary cerebral lymphoma has clearly decreased, changes in the frequency of other lymphomas are less well documented. At our institution, we recently noted that HIV-associated HD is becoming more frequent than HIV-related non-Hodgkin lymphoma (NHL). We have therefore analyzed the incidence of HIV-related lymphomas before and after the introduction of HAART in our single center series of 5,759 consecutive and unselected HIV+ patients (pts), followed by the Infectious disease department. The cutoff time was considered June 1997, when the use of HAART had become widespread among HIV+ individuals. Since 1985, 103 pts developed systemic aggressive NHL and 21 HD. The mean number of new HIV patients registered per year did not change over time (382 pre vs 326 post-HAART) as well as the overall number of HIV-related lymphomas per year (7.9 pre vs 9 post-HAART). The total number of HIV pts-years of follow-up was 24948, 17928 before and 7020 after HAART. Eleven and 10 cases of HD and 84 and 19 cases of NHL were diagnosed in the two periods, respectively. The mean incidence of HD/1000 pt/years rose from 0.61 to 1.42, with a rate ratio of 2.49 (p<0.038), whereas it decreased from 4.68 to 2.7 among NHLs (rate ratio 0.57, p=0.028). Within the limits of the size of the study, no significant differences were seen after the introduction of HAART in the demographic (HIV risk group, CDC category, previous AIDS) and clinicopathological features (stage, B symptoms, extranodal disease, histology) of HD and NHL. The only parameters showing significant changes were the number of patients presenting with CD4+ < 200/cmm (91% prevs 50% post-HAART; p=0.038) among HD patients, mean age at presentation (34 pre- vs 42 post-HAART; p<0.01) and, borderline, the mean CD4+ count/cmm (59 pre- vs 166 post-HAART; p=0.054) among NHL patients. In conclusion, in our consecutive and unselected series of HIV pts, a significant increase in the incidence of HD and a decrease of NHL have paralleled the introduction of HAART, with as yet little changes in the clinicopathological features of lymphomas. It may be speculated that the reconstitution of the immune system due to HAART may favour the development of those lymphomas, such as HD, with a rich lymphoid reactive compartment.

PO056
ACCURACY OF THE PHYSICAL EXAMINATION OF SUPERFICIAL LYMPH NODES IN LYMPHOMAS: A COMPARISON BETWEEN PALPATION AND ULTRASONOGRAPHY

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A careful physical examination, performed by a well-experienced clinician with special attention to the number and size of palpable lymph nodes, gives a necessary and sufficient evaluation of the superficial lymphatic sites. However, the increasing availability of ultrasonographic examination, that offers accurate measurement of the lymph node diameters, has led to increasing ultrasonographic check of superficial lymphadenopathies. Advantages of such a procedure are reported by higher accuracy when evaluating clinical response (based on the post-therapeutic variation of the sum of the products of the largest perpendicular diameters of all measurable lesions) and by accurate volumetric measurement of lymphadenopathies (with a potential contribution to the estimate of the total tumor burden). In 78 lymphomatous patients, observed since 1994, we compared the diameters of lymphadenopathies estimated through palpation by three well-experienced hematologists (E.A., E.M., P.G.) with the size of the same lymph nodes ultrasonographically measured by two well-trained operators (G.C.M., A.R.) with Sonolayer Toshiba 270SSA equipment connected to a 7.5 M Hz probe. We analyzed the regression of the products of the 2 perpendicular diameters (measured by palpation and ultrasonography) of each lymph node. Very satisfactory coefficients were recorded among lymph nodes of the cervical region (114 observations; R2=0.892) and of the inguinal site (28 observations; R2=0.800), while rather disappointing results were found in the analysis of lymph nodes of the supraclavicular region (34 observations; R2=0.360) and of the axillary one (55 observations; R2=0.529). Moreover, if we assimilate the shape of a lymph node to that of a rotation ellipsoid, the two shorter radii of which being equal, then the volume can be roughly drawn from the two available palpatory diameters. The regression between palpatory and ultrasonographic volumes gave the following results. cervical lymph nodes (114): R2=0.900; inguinal lymph nodes (28): R2=0.855; supraclavicular lymph nodes (34): R2=0.227; axillary lymph nodes (55): R2=0.286. Therefore, for the purposes of correctly evaluating clinical response or estimating total tumor burden, simple palpation is able to give very good linear and volumetric estimates only as far as cervical and inguinal lymph nodes are concerned, while it must be supported by ultrasonographic (or other radiologic) measurement when supraclavicular or axillary lymph nodes are involved.
PO057
EARLY-Stage, FAVORABLE Hodgkin's DISEASE: VERY GOOD RESULTS AND MINIMAL TOXICITY WITH VBMp CHEMOTHERAPY + INVOLVED FIELD RADIOTHERAPY

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In the management of early-stage, favorable Hodgkin’s disease low toxicity chemotherapy is often combined with radiotherapy to spare laparosplenectomy or reduce irradiation fields. The GISL demonstrated that VBM chemotherapy (vinblastine, VBL, bleomycin, BLM and methotrexate, MTX) combined with extended-field radiotherapy (EF-RT) is very effective in clinical stage I-IIA, non bulky patients (pts) with histologic types other than LD or NS (LD) [CO 1996:14: 527-33]. The subsequent GISL choices for the same subset of pts consisted in reducing RT to involved fields only and in modifying the VBM schedule with a 60% cut of the BLM dose in cycles 3 to 6 and oral administration of a fixed dose of prednisone (25 mg/die) for 5 days after every cytostatic drug infusion. The present study refers to 35 pts, aged 19 to 79, treated from 1997 to 1999, with a median follow-up of 28 months. Histologic type was NS in 18 pts, LP in 6, MC in 9, unclassifiable in 2. The mean number of cycles was 5 (3-6). Thirty-three pts reached complete remission (CR), two did not respond and then achieved stable CR with more aggressive regimens. Two pts, out of the 33 completely responders, relapsed, respectively, 6 and 19 months after the end of RT; the first reached CR again with ABVD chemotherapy, the second died of disease progression. The delivered dose intensity was very near the expected: 0.93 ± 0.12 for VBL, 0.92 ± 0.15 for BLM and 0.94 ± 0.11 for MTX. RT started 44 ± 25 days after the end of chemotherapy and the delivered total dose was 35.4 ± 3.0 Gy. Hematologic toxicity was negligible (apart from two pts aged > 70, who experienced a grade 3 and 4 neutropenia and thrombocytopenia). Pulmonary toxicity was very low (grade 1 in 2 pts only) and the neurologic one was mild (grade 1 in 5 cases, grade 3 in one). A grade 2 and 3 liver toxicity was recorded in 2 pts, likely related to MTX and it quickly recovered without specific therapy. These results suggest that the combination VBM p+EF-RT, with reduced irradiation and lowered BLM dose, demonstrates very low toxicity without worsening of the clinical outcome obtained with the preceding VBM +EF-RT therapeutic program.

PO059
VIDEO-LAPAROSCOPIC SPLENECTOMY IN HEMATOLOGY: EXPERIENCE ON 130 CASES

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At present, video laparoscopic (VL) splenectomy is the gold-standard for the treatment of the majority of hematological splenic diseases. Our experience, started in 1995, has reached the considerable number of 130 operations. At first, VL was only used for idiopathic thrombocytopenia (ITP), later it was applied in all hematologic diseases (hereditary spherocytosis, beta thalassemia, autoimmune hemolytic anemia, lymphoproliferative diseases, idiopathic myelofibrosis, thesaurysmosis, primary hypersplenism). Initially, the presence of a large spleen was a contraindication for VL, but now, with the new surgical tech-
In spite of progress made in treatment of advanced Hodgkin’s disease (HD), 30-35% of patients still cannot obtain a complete remission (CR) or relapse within the first three years from diagnosis. The International Prognostic Score (IPS) identifies 2 groups of patients with advanced HD at different risk of progression or relapse. However IPS cannot identify patients with very poor prognosis. The aim of this study was to define whether serum levels of the soluble form of the CD30 molecule (sCD30), already demonstrated as an independent prognostic factor for HD, may improve the IPS and identify high-risk patients eligible for more intensive treatment. We analyzed the long term outcome of 101 patients with advanced HD (Ann Arbor stage I-II B or bulky, III and IV) observed from January 1981 to June 1997 in our Institution. They were 49 males and 52 females. The median age and median follow-up were 30 years (range 15-65) and 90 months (range 18-187), respectively. Histologic subtypes were: LP in 3 patients, NS in 74, M C in 22, unclassified in 2. Disease stage was: I in 4 patients, II in 38, III in 32 and IV in 27. Bulky disease was present in 38 cases and B symptoms in 59. All patients received chemotherapy: 68 MOPP/ABVD and 33 ABVD; 59 cases received adjuvant radiotherapy. Ninety-six patients achieved CR. Freedom from progression of disease (FFP) and overall survival (OS) at 8 years were 72% and 86% respectively. The 8 years FFP of 68 patients with IPS =2 was 78% vs 60% (p=0.03) of 33 patients with high IPS. OS of the two group was 90% vs 77% respectively (p=n.s.). Sixty patients had sCD30 <100 U/ml and 41 ≥100 U/ml: FFP of the two groups was 83% and 57% (p=0.005) and OS 89% and 80% (p=0.04) respectively. Forty-five patients had sCD30 <100 U/ml and IPS ≥2, 38 had sCD30 ≥100 U/ml or IPS ≥2 and 18/101 (17.8 %) sCD30 ≥100 U/ml and IPS >2. FFP of the three groups was 84%, 72%, 44% respectively (p=0.01) and OS 85%, 97% and 61% (p=0.01). In our study the combination of IPS ≥2 and sCD30 serum levels ≥100 U/ml identifies a subgroup of advanced HD patients with FFP < 50% in whom intensive therapy could be proposed as up-front treatment. However, the clinical impact of this combination of prognostic factors in HD needs to be validated in larger series.

Background. Chemotherapy is believed to be the best therapeutic choice for early stage HD patients with unfavorable prognostic factors. Main concerns regard both relapse rate and long term therapy related toxicity. In order to reduce relapse rate, and minimize long term side effects, combined modality programs (brief chemo + radiotherapy) could be a suitable approach. Design and methods. The GISL planned a prospective controlled multicentric study to investigate the effectiveness and safety of 4 cycles ABVD plus RT involved field (40 GY) in treatment of unfavorable early stage HD. (stage I-II B; I-II A bulky; and IIIA except for LD histology). This study enrolled (Dec. 1991-June 1997) 105 untreated HD patients: 7 (6.7%) in stage I; 71 (67%) in stage II; 27 (26%) in stage III A; 27% had bulky disease 69% mediastinal enlargement and 18% had B symptoms. The median age was 32 years (15-66) and 42 (40%) were male. The histology was: NS 58%, MC 28.6%, LP 4%; LD 0.9%. Results. After four cycles of ABVD we obtained 83 (82%) CR and 17 (16.8%) PR. After RT the number of CR raised to 92. Three pts died, two of disease progression, one of secondary neoplasm (LAM), 12 (11%) pts relapsed, 6 (22%) in stage III. Seven out of 12 (58%) relapsed pts had an IPFI score > 3. With a median follow-up of four years, five years O.S. is 95% and RFS is 93% for pts in stage I-II and 88% for pts in stage III respectively. Conclusions. Four cycles of ABVD followed by adjuvant radiotherapy produced in our experience high O.S and RFS rates with negligible early toxicity. Pts with IPFS score > 3 or stage III could benefit of a more intensive therapy. Long term follow-up is necessary to ascertain the long term safety of this approach.

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PO062
PROGNOSTIC VALUE OF INTERACTION AMONG VARIABLES IN THE EVALUATION OF OUTCOME IN HODGKIN'S DISEASE

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Great effort has been made in the last 15 years in the attempt to build an efficient prognostic model of HD. However even the last attempts both derived from the huge data set of the IDHD, (IPFP: Hasenclever D. NEJM 1998; 339: 1506-15; IDHD: Gobbi PG, Haematologica 1994; 241-55) cannot be considered complete, as the authors themselves have stated. The prognostic role of interactions among variables although clinically and biologically plausible has not been statistically confirmed yet. However the existence of such interactions could contribute to explain the partial reliability of prognostic models developed so far. We therefore have systematically investigated the importance of conjoin effects of the 7 prognostic factors taken into account by the IPFP (age, albumin, Hb, sex, lymphopenia, leukocytosis, stage, all categorized as binary variables) and by the IDHD (age, albumin, Hb, histology, B symptoms, stage, involved area distribution) on the outcomes of 494 HD patients belonging to the GISL database. Initially we fitted Cox proportional hazard model considering each set of the IPFP and IDHD model variables to estimate OS and TTF of ours series and we have estimated parameters that link each variable to the endpoints. Then within each of the two groups of factors we have investigated if some interactions between variables might have a significant prognostic value. For the interaction between albumin and stage added a significant improvement in data fitting (p < .034) using IDHD factors to estimate OS as the same did the interaction between age and stage (p < .016) when the IPFP set of factors were considered to estimate the TTF. We conclude that some interactions among variables, at least in the GISL database, have a sizeable prognostic impact on the main outcome endpoints and they could be useful in modelling new prognostic scores and could contribute to explain the partial reliability of prognostic models developed so far.

PO063
PROGNOSTIC EVALUATION OF BELOW DIAPHRAGMATIC HODGKIN'S DISEASE (BDHD). COMPARISON OF TWO PROGNOSTIC MODELS DERIVED FROM IDHD DATA BASE

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Background. Hodgkin's lymphoma rarely occurs below diaphragm (BDHD). Although it is widely recognized that this rare occurrence is frequently associated with well known adverse prognostic factors it is believed to behave as the above diaphragm disease occurrence (ADHD). While some of the prognostic indexes recently proposed have shown themselves to be applicable to ADHD there are no data concerning BDHD. Purpose: To test if one of two different prognostic models or both derived from the large data set of the IDHD (IPFP: Hasenclever D. NEJM 1998:339:1506-15; IDHD: Gobbi PG, Haematologica 1994, 241-255) are able to predict the outcome of a series of BDHD; to verify if the two BDHD subsets (with or without peripheral lymphonodes) have a different prognosis and if it is consequently worthwhile to maintain them separately. Patients and methods. The outcome (TTF-OS) of 47 BDHD patients enrolled at GISL centres (June 1982 - December 1995; median follow up 5.8 years) was compared with both the expected median survival estimated by the IDHD parametric hazard model and the risk groups generated by IPFP score. Results. The series showed a rather high rate of unfavourable prognostic factors and accordingly the outcome has been worse than expected for an early stage HD series (observed TTF and OS respectively 61% and 64% at 10 years). IDHD divide this selected series of early stage HD in groups with significantly different prognosis (p < .02). In accordance with IPFP score over 50% of the patients had 3 or more unfavourable factors; however the IPFP index does not separate the series into subgroups with statistically different prognosis probably as result of the small size of the sample. The differences in OS and TTF that we have observed in patients with disease exclusively confined to the abdomen (C) in respect to those with peripheral lymph nodes involvement (P) were statistically significant (P vs C: OS p<.05 TTF p<.03). Moreover both indexes correctly predicted the worse outcome as regard (C) BDHD and (P) BDHD groups: expected OS predicted by IDHD (p<.02) and IPFP scores (p<.05) between (P) and (C) subgroups were significantly different. Conclusions: Our data indicate that the performances of both indexes are rather far from those shown in the original reports when applied to this rare subset of patients. However both indexes agree in identifying BDHD with only intra abdominal lymphonodes as a high risk failure group. Although the IDHD model better predicts the outcome in terms of OS, IPFP is easier to use and more suitable for daily clinical application.

PO064
PRELIMINARY RESULTS OF THE ITALIAN LYMPHOMA INTERGROUP RANDOMIZED STUDY ABVD VERSUS EVE IN UNFAVOURABLE STAGE I A AND II A HODGKIN'S DISEASE


Background. A high cure rate has been reported in unfavorable stage I and II Hodgkin's disease (HD) with ABVD plus extended field radiotherapy. However late cardiac and pulmonary toxicities are often induced by the association of mediastinal irradiation and bleomycin plus adriamycin administration. In 1990 the EVA regimen was proposed as an effective and low toxic salvage regimen for HD. To test in this set of patients the benefits of the new EVE regimen, in terms of lung and heart toxicity, in comparison to the conventional ABVD.
schedule. Patients and Methods. From 1996 to 2000, the patients affected by unfavorable stage I and II A HD were enrolled into the study. The inclusion criteria were age between 18 and 65 and stage I or II A HD with at least one of the following unfavorable prognostic factors: bulky disease, involvement of more than 3 lymph node areas, E lesion, ESR higher than 40, hilar involvement, infra-diaphragmatic presentation. The patients were randomized at diagnosis to receive 4 courses of either ABVD or EVE regimens. At the end of the chemotherapy program, the patients underwent an involved field radiotherapy 36 Gy. The EVE schedule was as follows: Epirubicine iv. 70 mg/m² on day 1, vinblastine iv. 6 mg/m² on day 1, etoposide iv. 100 mg/m² on day 1 followed by etoposide os 150 mg/m² on days 2 and 3. The cycle was repeated every 21 days. The patients were classified on the basis of the response as being in: complete remission (CR), unconfirmed complete remission (CRu), partial remission (PR) and non response (NR) according to the Cotswolds meeting criteria. Results. One hundred and eighty nine patients were randomized: 96 and 93 for the ABVD and the EVE arms respectively. The distribution of patients for stage was 9% in stage I and 91% in stage II. One hundred and fifty four patients are so far evaluable for toxicity and response. Both regimens were well tolerated in terms of acute toxicity. No significant extra-hematological toxicity has been reported. Forty eight patients, mainly in the AVBD arm had at least one episode of neutropenia grade 3 or 4, without any septic complication. The final remission rate as expressed by the association of RC and RCu is not different between the ABVD and EVE arms (91% vs. 85%, p=ns). However the relapse rate in patients treated with EVE is significantly higher than that of patients treated with ABVD (12% vs. 1%, p<0.01). The relapsed patients have been so far rescued with a second line chemotherapy treatment. The 4-year overall actuarial survival rate is 92% with no difference between the two arms. Conclusions. In the EVE arm a relapse rate higher than in the ABVD one has been demonstrated. The preliminary results of this study suggest that in unfavorable stage I and II HD a treatment strategy less intensive than 3 or 4 ABVD plus involved field radiotherapy is associated with a high relapse rate.

P0065
PRELIMINARY RESULTS OF THE VEPEMB CHEMOTHERAPY REGIMEN IN ELDERLY HODGKIN’S DISEASE PATIENTS: AN ITALIAN LYMPHOMA INTERGROUP STUDY

On behalf of the Italian Lymphoma Intergroup

Aim of the work. To evaluate the results of a new non aggressive chemotherapy regimen (VEPEMB) designed for elderly Hodgkin’s disease (HD) patients. Patients and Methods. Between 1995 and 2000, 92 HD patients over 65 years were staged and treated from 27 different institutions according to the VEPEMB schedule: vinblastine 6 mg/m² day 1, cyclophosphamide 500 mg/m² day 1, procarbazine 100 mg/m² days 1 through 5, prednisone 30 mg/m² days 1 through 5, etoposide 60 mg/m² os days 15 through 19, mitoxantrone 6 mg/m² day 15, bleomycin 10 mg/m² day 15. The regimen was scheduled every 28 days. Forty four low risk patients (IA and IIA) entered a program of 3 courses of VEPEMB followed by involved field irradiation. Forty eight high risk patients (stage IIb-IV) entered a program of 6 courses, with radiotherapy limited to the bulky areas. Results. Mean age was 71 (range 66-83). The patients were distributed according to stage as follows: 17 (19%) in stage I; 34 (37%) in stage II; 26 (28%) in stage III; 15 (16%) in stage IV. B symptoms were present in 35 patients (38%). Comorbidity was present in 29 patients (31%). Seventy eight patients (39 low risk and 39 high risk) are so far available for toxicity and response. The tolerance to the first three courses was good. Seven out of the thirty nine high risk patients (18%) needed a subsequent plan modification and/or interruption (course fourth to sixth) for poor tolerance and/or toxicity. Patients treated with six courses experienced at least one episode of grade III-IV neutropenia, but hospitalisation for fever was seldom required and no toxic death was observed. As expected the outcome was highly affected by stage: CR rate 100% in low risk vs. 65% in high risk (p<0.01); Relapse Free Survival 100% vs. 64% (p<0.01); Event Free Survival 82% vs 40% (p<0.01). The Event Free Survival was non affected by age itself (66-70 years 69%; 71-75 years 60%; ≥ 76 years 53%; p=ns), while it was highly affected by comorbidity (no 79%; yes 44%, p<0.01). Conclusions. The preliminary results of the VEPEMB regimen in elderly Hodgkin’s disease patients are encouraging. It is a well tolerated and effective regimen. A randomized study in order to compare VEPEMB to ABVD in this set of patients in terms of toxicity and final event free survival should be useful.

P0066
TREATMENT OF ADVANCED HODGKIN’S DISEASE WITH A MODIFIED STANFORD V REGIMEN

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Cure rates for patients (pts.) with Hodgkin’s disease in an advanced stage treated with conventional chemotherapy, mostly the ABVD regimen, range between 50 and 60%. The recent introduction of intensified chemotherapy regimens may improve the prognostic outlook for these pts at the costs of an increased rate of toxicity. First data are emerging indicating a higher incidence of secondary myelodysplasias and leukemias. We initiated a phase II protocol evaluating the toxicity and efficacy of a modified Stanford V regimen. The treatment consisted of 3 four-week cycles of a weekly alternating polychemotherapy including cyclophosphamide (substituting the more leukemogenic chlorambin), Adriamycin, vinblastine, vincristine, bleomycin, and etoposide. Local radiotherapy was planned for patients with initially bulky disease. So far, we included 27 pts. The median age was 32 years (range 18-65 years). Sixteen pts were male and 11 were female. Nine pts presented with bulky stage II disease, 10 pts with stage III, and 5 pts with stage IV disease, while 3 pts were included for high-risk non-bulky stage II disease. B-symptoms were present in 19 pts. Twenty patients completed the treatment and are available for evaluation. Toxicity was mild, and mainly hematol-
PO067

EPIDEMIOLOGIC ASPECTS OF HEMATOLOGICAL MALIGNANCIES IN SARDINIA, 1974-1993

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We have collected all cases of hematologic malignancies (HM) newly diagnosed in the resident population of Sardinia in the period 1.1.1974–31.12.1993. Cases were searched through a personally done examination of all registers of all medical departments and of all pathology institutions operating in that time in Sardinia; confirmation of diagnosis, date of diagnosis and demographic data of patients were obtained through a personally done consultation of their clinical records. Epidemiologic evaluations have been done according to the recommendations of dos Santos Silva. A total of 7339 cases were found. Sex and age distribution documented that HM increase with increasing age, with a male predominance in all age groups. The same conclusions are evident evaluating age- and sex-specific incidence rates. Mean age at diagnosis was 55.6. Male-to-female ratio was 1.36:1. Crude annual incidence rate of HM rose statistically during the period covered by our survey. Mean incidence per year for the whole period is 367 (range 214-559; median 358) but was 274 (range 214–331; median 251) in the first ten years and 460 (range 351–559; median 460) in the second ones. This increase is not due solely to ageing of Sardinian population; indeed it is evident also in the curve of age-standardized annual incidence rates (standard: Sardinian population at 1981 census). We cannot, at the moment, evaluate which is the contribution of a real increase of incidence and that of an increase of diagnosis in previously undetected patients (due to increased physician awareness of diseases, more available clinical and laboratory facilities, etc.). We think that nowadays in our island hematological institutions have to cope with about 600 new cases/year of HM; of them about 200 are more than 65 years old. This makes necessary: 1) to adapt hematological structures to increased needs; 2) to take into account, also in the professional training of their staff, the problems deriving from increasing age of hematologic patients.

References:

A multi-disciplinary approach to the onco-hematologic patient. 

The case, with its unusual presentation along with atypical pathology features was brought to the weekly clinico-pathologic conference at our Institution. There was a general consensus on the opportuneness of performing splenectomy in this patient, owing to the considerable risk of undergrading this lymphoma on BMB and to the frequent evolution of the NLPHD into a more aggressive B-cell neoplasm. A laparoscopic splenectomy was performed and the diagnosis of NLPHD was confirmed on spleen and hepatic tissues, but multiple foci of DLBCL were additionally discovered in enlarged hilar splenic lymph nodes. The patient was treated with an aggressive chemotherapy regimen (VACOP-B) followed by high-dose of cyclophosphamide, peripheral stem-cell harvest and autologous transplantation after BEAM high-dose regimen. At ten months following autologous transplantation the patient is alive in complete remission.

We have reviewed 144 patients treated in our department for Hodgkin's disease from 1980 to 2000. Mean age was 38.115 patients had a stage I disease, 75 stage II, 29 stage III and 25 stage IV. Seventy-nine percent of patients achieved a complete remission. We considered the following late toxicities: cardiac, pulmonary, endocrinal and gonadic, second malignancies. Eighteen percent of patients presented a cardiovascular disease (6 myocardial infarction, 5 pericarditis, 5 heart failure, 15 important arrhythmic disorders). Lung toxicity was represented by a symptomatic or asymptomatic fibrosis. It affected 28.8% of patients treated with radiotherapy vs. 8.3% of patients who did not receive it (p<0.001). Fibrosis appeared also in some patients (7) who received only chemotherapy; it always contained bleomycin. Three patients presented both fibrosis and, after few years, pneumo- orax. We observed 8 second cancers (5.53%); 4 hematologic and 4 solid cancers. The interval between treatment for HD and the outbreak of cancer ranges beyond 2 to 12 years. Four patients died for these pathologies; the patients with non-lymphoid leukemias (2) were treated with MOPP/ABVD and they did not reach CR. Most cases of endocrine diseases were represented by clinical or subclinical hypothyroidism (6 patients). They all underwent radiotherapy on the neck. Two patients developed diabetes mellitus; now they take oral therapy after a phase of insulin therapy. We lack many pre-therapy laboratory datas, especially those regarding male infertility before diagnosis of HD. So we cannot confirm what literature says about sub-fertility in male with HD. Four female patients report spontaneous abortion in first quarter of pregnancy; they all received chemotherapy (MOPP/ABVD) and radiotherapy. Finally, our study supports what is coming up by all international trials: patients who recover from HD present a high incidence of consequences linked to treatment. Our study confirms that pts cured from HD suffer a high incidence of late toxicity. New polychemotherapy regimens (BEACOPP and Stanford V) are designed to achieve higher rates of CRs with first induction therapy, lowering the risk of relapse and the necessity of second-line therapies with or without radiotherapy. The less frequent use of alkylating agents and of combined therapy should reduce the risk of second malignancies.
PO071
GLUTEUS MUSCLE LOCALIZATION OF HODGKIN’S DISEASE: A CASE REPORT

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Gluteus muscle involvement by Hodgkin’s disease (HD) is uncommon. To the best of our knowledge such localization has been reported only in two cases. We describe the case of a 31-year old woman affected by HD, previously heavily treated, that developed involvement of gluteus muscle two years after diagnosis. Hodgkin’s disease, scleronomodular type, stage IV A, was diagnosed in April 1997, when the patient developed diffuse adenopathies, splenomegaly and bilateral basal nodular lesions of the lung. Bone marrow biopsy was negative. In June 1999, after three lines of chemotherapy, the patient showed disease progression because of reappearance of adenomegalies, bone marrow involvement and appearance of a solid lesion (51 × 41 mm) in the left gluteus muscle. TC showed massive enlargement and biopsy confirmed the lymphomatous nature of such lesion. The lesion, in spite of further chemotherapy, increased volume and determined sciatalgic symptoms. For this reason the patient underwent PBSC mobilization and harvesting and autologous bone marrow transplantation using BEAM chemotherapy. After high dose chemotherapy 50% volume reduction of gluteus lesion and very good response to conventional chemotherapy as well as to High Dose chemotherapy.

PO072
AN UNUSUAL RELAPSE OF HODGKIN’S DISEASE AFTER 13 YEARS OF COMPLETE REMISSION

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We present what is to our knowledge the first case of relapse of Hodgkin’s disease with intracranial lymphoepitheliopathies. Case Report. On June 2000, a 44-year old man with a six-months history of progressive occipital headache, vertigo, right trigeminal neuralgia, dysphagia and throat irritation, was admitted to our hospital. At 1983 the patient developed a nodular sclerosis Hodgkin’s disease. The clinical and pathological (splenectomy) stage were IIA. He was treated with exclusive mantle radiotherapy obtaining complete remission. In 1987 he had a myocardial infarction. On admission, physical examination demonstrated some right latero-cervical lymph nodes. The hematologic and chemical parameters were normal. A total body CT showed right latero-cervical, jugular foramen, hypoglossal canal and posterior fossa lymphoepitheliopathies compressing the cerebellar tonsil, cervical spinal cord and medulla oblongata. A biopsy of one latero cervical lymph node revealed a relapse of nodular sclerosis Hodgkin’s disease (BNU type I). A bone marrow biopsy was negative lymphoma involvement. The stage was defined II A. Therapy was instituted with 6 cycles of EBVD regimen chemotherapy given every four weeks. The EBVD regimen was vincristine 6 mg/m² days 1 and 15, dacarbazine 375 mg/m² days 1 and 15 days, bleomycin 10 U/m² days 1 and 15, epirubicin 35 mg/m² days 1 and 15. The epirubicin was chosen for less cardiotoxicity. No hematologic and extra-hematologic toxicities occurred unless complete alopecia. At one month after therapy, a CT scan was performed showing a complete remission. Chemotherapy was followed by involved-field radiotherapy. Three months later the patient presented clinical remission. Actually he is still in good health. Hodgkin’s disease is one of neoplasms with the highest curability rate. In particular, early-stage patients achieve long-term survival rates as high as 75-90%. On the other hand, early-stage Hodgkin’s disease, treated with radiotherapy alone, often present lymph nodes and extranodals relapses few years after therapy in 20-40% of cases. Some authors report statistically significant improvement in relapse free survival adding chemotherapies to radiotherapy in comparison with radiation therapy alone. However the incidence of secondary neoplasms is increased. Long term follow-up will be necessary to document the role of the different therapeutic protocols. In conclusion, the particularity of our case is very long disease free interval and above all unusual lymph node recurrence.

PO073
DEXA-BEAM IS EFFECTIVE IN POOR RISK HODGKIN’S DISEASE RESULTING IN HIGH RATE OF PERIPHERAL BLOOD STEM CELLS MOBILIZATION

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The dea-BEAM regimen (dexamethasone 8 mg every 12 hours iv from day 1 to 10, carmustine 60 mg/m² iv on day 2, melphalan 20 mg/m² iv on day 3, Etoposide 75 mg/m² iv and cytarabine 100 mg/m² iv every 12 hours from day 4 to 7) was administered to 15 patients (pts) with advanced Hodgkin’s disease (HD). The aims of the study were: 1) to assess the efficacy and toxicity of the regimen as salvage therapy; 2) to test its capability of mobilizing CD34 positive (CD34+) peripheral blood stem cells (PBSC); 3) to verify the percentage of patients who are eligible for autologous stem cell transplantation (auto-SCT) following dea-BEAM administration. From 1995 to 1999 15 patients were enrolled into the study. There were 6 males and 9 females, with a median age of 29 years (range: 14-57). At diagnosis, 2 patients were in stage II, 10 in stage III and 3 in stage IV, respectively and all received treatment with conventional ABVD. 9 were resistant, 1 showed progression of disease, 4 had early relapse (median 7 months, range 3-9) and 1 was in second relapse. At relapse, 4 pts had extra-nodal involvement. All pts were given 2-4 courses of dea-BEAM, depending on response after the first course and toxicity. Five pts (34%) achieved complete remission (CR), 6 (40%) partial remission (PR), 2 (13%) failed to respond and 2 (13%) died for severe sepsis during the prolonged hypoplasia induced by chemotherapy. In a further patient, severe respiratory sepsis, requiring admittance to intensive care unit occurred.
and resolved at hemopoietic recovery. All patients experienced grade IV alopecia. Grade IV neutropenia and thrombocytopenia occurred in all patients, the median time for neutrophil and platelet recovery to > 1000/cmm and > 50000/cmm being 18 days and 20 days, respectively. 12/15 pts (80%) were evaluated for PBSC mobilization after dexa-BEAM. In 10/12 (83%) a successful PBSC mobilization was observed with sufficient collection of CD34+ cells. The peak number of circulating CD34+ cells was reached at a median of 20 days from the start of therapy (range: 15-26). A median number of 9.4 ×10^6/kg (range: 4.1-40) CD34+ was collected. Among mobilizers, 9 pts (75%) did actually receive auto-PBSC. In particular, 4 of them achieving CR after dexa-BEAM, were given MOPP and then auto-transplant-ed after conditioning with BEAM. 3 BEAM conditioned pts are still in CR at 13, 17 and 9 months, respectively, while 1 showed early relapse post-ASCT. Among BVC conditioned pts, 2 are still in CR at 15 and 14 months, 2 did never reach CR but are alive in PR and 1 died after relapsing. We conclude that dexa-BEAM is effective as salvage treatment in advanced HD with high rate of PR and 1 died after relapsing. We conclude that dexa-BEAM, were given MOPP and then auto-transplant-ed after conditioning with BVC. 3 BEAM conditioned pts are still in CR at 13, 17 and 9 months, respectively, while 1 showed early relapse post-ASCT. Among BVC conditioned pts, 2 are still in CR at 15 and 14 months, 2 did never reach CR but are alive in PR and 1 died after relapsing. We conclude that dexa-BEAM is effective as salvage treatment in advanced HD with high rate of PR and 1 died after relapsing."

**PO074**

**HODGKIN’S DISEASE FOLLOWING CHRONIC LYMPHOCYTIC LEUKEMIA**


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High-grade non-Hodgkin’s lymphoma occasionally develops after a diagnosis of chronic lymphocytic leukemia (Richter’s syndrome). More rarely, large tumor cells have the morphological and immunophenotypic features of classical Hodgkin and Reed-Sternberg (H-RS) cells. We report a case of CLL that developed Hodgkin’s lymphoma (HD) after a 10 year slowly progressive disease treated with single-agent chemotherapy. The patient was a 76 year old man admitted to hospital in August 1991 at the age of 66, because of left inguinal and bilateral cervical lymphadenopathies and lymphocytosis. White blood cell count was 21×10^9/l with 83% lymphocytes. Bone marrow biopsy showed 60% diffuse infiltration by small lymphocytes. Flow cytometry immunophenotyping of peripheral and bone marrow blood revealed the presence of an abnormal CD10-, CD5-, CD23- small lymphoid cell population. Histology of a right cervical lymph node diagnosed diffuse infiltration by small lymphocytes. Thoracic and abdominal CT scans were normal. During 10 years, the patient received 22 cycles of chlorambucil and prednisone (0.4 mg/kg and 1 mg/kg daily, respectively, for 4 days every month) with satisfactory results. Lymphadenopathies disappeared and lymphocytosis was reduced. Of-therapy from June 2000, the patient presented in November 2000 with bilateral inguinal and axillary lymphadenopathies. Histology of axillary lymph nodes revealed the presence of typical CD15+, CD30+ Reed-Sternberg cells. Bone marrow histology showed a normal percentage of lymphocytes and focal infiltration of Reed-Sternberg cells. Thoracic and abdominal computed tomography scans were normal. The patient is now receiving chemotherapy according to the ABVD regimen. Many cases of HD complicating B-CLL have been reported so far. Although CLL and HD are considered two distinct lymphoproliferative disorders, recently some authors have speculated that HD and CLL derive from the same clone (Blood, Vol 95, No. 3, 2000: pp. 1023-31). Supporting this hypothesis lies the B-cell origin of HD, which is now a matter of fact. However, the possibility of a second tumor, favored by the assumption of alkylating agents, cannot be ruled out, particularly in cases showing a long interval between the two diagnoses.

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**PO075**

**HCV, AUTOIMMUNITY AND LIMPHOPROLIFERATION: A CASE REPORT**

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The main clinical characteristic of HCV is the capacity to determine, more often, persistent infection escaping from the immune response. Chronic immune activation, that control viral replic-ations, determines the appear of various diseases, not always linked to liver disease. HCV can determine a series of extrahepatic disease, always of immunomediated pathogenesis. Moreover, more recently many data show an elevated prevalence of HCV in lymphoproliferative disease (LPD). Many authors think that the chronic persistence of HCV in lymphocytic cells can determine oncogenic mechanism that can cause some lymphoproliferative disease. Now we report a case of a patient affected by Hodgkin’s lymphoma, autoimmune hemolytic anemia during HCV. Clinical case. Patient L.G., 51-year old, reported anemia, fever, weight loss and red urine. We saw splenomegaly, adenomegaly and the analy-sis showed an autoimmune hemolytic anemia, moreover HCV tests including HCV RNA resulted positive. The bone marrow aspiration and biopsy showed only increased erythropoiesis. CAT showed a mediastinal mass and many lymphnodes. Lymph node biopsy showed Hodgkin’s lymphoma nodular sclerosis stage I. We started corticosteroid therapy with progressive normalization of hemochromocytometric parameters, after the patient started chemotherapy ABVD for 6 cycles followed by mediastinic radiotherapy high energy 30 Gy in total. After this program he reached complete remission and we stopped therapy. The patient is now in good health and he is followed in our day hospital. Conclusions. Viruses determine many autoimmune diseases, infact viral infec-tions cause alteration of immunologic regulation and of lymphoproliferation. It seems that HCV can determine a lymphomagenesis on B Lymphocyte, moreover viral proteins can interfere with apoptosis and can cause a genomic instability. This can determine multiple mutations of oncogenes and a malignant irreversible trasformation. We think that an important valuation of immunologic alterations and of lymphatic system is necessary in the presence of HCV.

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**CD34⁺ Cells from Paroxysmal Nocturnal Hemoglobinuria Patients Are Deficient in Surface Expression of Prion Protein**

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Prion protein, in its normal form (PrPc), is a rather ubiquitous GPI-linked molecule of unknown function. The presence of PrPc appears to be necessary for accumulation of PrPsc (the pathologic form of prion) in the neurodegenerative diseases such as Creutzfeldt-Jacob and Gerstmann-Sträussler-Scheinker. In addition to cells of the central nervous system, PrPc also is constitutively expressed on hematopoietic progenitors and some of the mature blood cells such as erythrocytes, lymphocytes and platelets, which may be involved in dissemination of PrPsc into the CNS. Paroxysmal nocturnal hemoglobinuria (PNH) is a rare hematologic disease due to somatic mutation in PIG-A gene. Its product is required for the first step in the biosynthesis of glycosylphosphatidylinositol (GPI) anchor. Consequently, in PNH, progeny blood cells derived from the affected stem cell lack from their surface all GPI-anchored proteins. In addition to the cardinal symptom of hemolysis, PNH is strongly associated with idiopathic aplastic anemia. Because PrPc is a GPI-linked protein, we have studied prion expression on cell lines and hematopoietic primary progenitor cells from normal individuals and PNH patients. Using Affimetrix genechip array we found that prion mRNA is expressed in highly purified normal CD34⁺ cells. Prion expression also was confirmed by flow cytometry analysis: intact PrPc is constitutively present on normal CD34⁺ cells, and its expression appears not to be modulated by various in vitro stimuli, including IFN-γ, Fas-L and TGF-β. That prion is expressed on hematologic progenitor cells as a GPI-linked protein was demonstrated using phospholipase C (PPLC) digestion. In the bone marrow from PNH patients, two distinct populations of CD34⁺ cells were detected based on the expression of PNH indicator proteins CD55 and CD59: CD34⁺ cells with PNH phenotype (CD55⁻/CD59⁻) which did not express PrPc; and phenotypically normal CD34⁺ cells (CD55⁺/CD59⁺) with prion expression similar to those seen in normal CD34⁺ cells. In analogy to primary hematopoietic cells, prion expression was detected in wild type K562 cells but not in PIG-A mutant counterpart. When the accumulation of prion was studied by flow cytometry, both wild type and mutated K562 showed comparable amount of intracellular prion protein; these results were also confirmed by immunoprecipitation. In conclusion PNH positive CD34⁺ cells and their progeny are deficient in prion membrane expression. Pathophysiological consequences of the lack of prion expression remain unclear.

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**Table: Follow-up of CD34⁺ Cells from Paroxysmal Nocturnal Hemoglobinuria Patients**

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<th>Pt/µL</th>
<th>Tran.</th>
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</table>

Up to now 34 cases of PNH treated with ALG have been reported and 23 had a favorable response; response duration is often unreported. All the reports, including the present, suggest the utility of immunosuppressive therapy in hypoplastic PNH, even if it produces improvement but not cure of the disease.

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**PO076**

**Immunosuppressive Therapy in Paroxysmal Nocturnal Hemoglobinuria**

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Paroxysmal nocturnal hemoglobinuria is a rare hematological syndrome due to the emergence of a haemopoietic clone affected by a mutation in the PIG A gene and characterized by intravascular haemolysis, progressive bone marrow hypoplasia and thrombophilia. The clonal progeny lacks the glycosyl phosphatidylinositol (GPI) anchor to the synthesis of which the altered gene participates. Deficiency of this structure on cell membrane causes the absence of several proteins leading to various cell dysfunctions, among which the best known is intravascular haemolysis. The mechanism that produces bone marrow failure remains still unknown: in these years a possible role for autoimmunity has been claimed, and immunosuppressive treatments have been given to hypoplastic PNH. Here we report results concerning immunosuppressive therapy in 4 PNH patients whose median age was 30 yrs; they were all transfusion-dependent and in peripheral blood GPI protein deficiency was estimated by cytofluorimetric investigation in more than 50% of granulocyte and monocyte. Patients underwent a course of antilymphocyte globulin (ALG) 0.5 mL/kg/ day for 5 days, Prednisolone (PD) 1 mg/kg/day for 9 days and Cyclosporin A (CyA) 500 mg day for the following six months. Patients 1 and 2 are still taking CyA and PD in reduced doses; patient 4 after 6 years underwent a second course of ATG and has being taking CyA and PD in reduced doses; patient 4 after 6 years underwent a second course of ATG and has being taking CyA and PD in reduced doses; patient 4 after 6 years underwent a second course of ATG and has been taking CyA and PD for 3 years. We noted a prolonged response to therapy in all treated patients; 3 of them do not need transfusion therapy anymore. Granulocyte counts are improved in all patients, without any change in the percentage of GPI deficient cells.
RESOLUTION OF WARM AND COLD AGGLUTININ DISEASE NOT ASSOCIATED WITH LYMPHOMA AFTER TREATMENT WITH RITUXIMAB

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Rituximab, the anti CD20 antibody, used for refractory/relapsed follicular lymphoma of B cell origin, has shown promising activity in the treatment of some autoimmune disorders related to a B-cell clone. In particular three cases of cold agglutinin disease (CAD) are reported in the literature, refractory to the standard therapy, that have resolved after rituximab treatment. We report the case of a 68 year old Caucasian woman, referred to our clinic because of weakness of one week and dyspnea. Her medical history was unremarkable. Physical examination revealed pallor, slight scleral jaundice, but neither lymphadenomegaly or abdominal organomegaly. At admission laboratory investigations showed severe normochromic normocytic anemia (hemoglobin level of 6.6 g/dL and a hematocrit of 16.3%), reticulocyte count was 12.3%. The white blood cell (WBC) count was 4600/µL with neutrophils 69%, lymphocytes 23% and monocytes 2%. The platelet count was 277000/µL. Biochemical evidence of hemolysis was supported by: bilirubin level of 1.5 mg/dL, lactate dehydrogenase (LDH) of 2626 IU/L and haptoglobin below 7 mg/dL. The direct antiglobulin test was positive for polyvalent serum (+++), complement (+/-) and IgG (+++). Cold agglutinins of IgM type had a titer of 1:1024. Serology for human immunodeficiency virus (HIV), hepatitis B and C and Mycoplasma pneumoniae were negative. Total body iron studies showed severe iron deficiency (serum iron, total iron binding capacity, iron saturation less than 5% of total cellularity), as evidenced by immunophenotyping study. Bone marrow molecular study showed weak positivity for clonal rearrangement of the immunoglobulin heavy chain gene by polymerase chain reaction (PCR). Prednisone therapy was started as soon as possible at a dose of 1 mg/kg/daily. Hemoglobin level rose up to 12.8 g/dL, concomitant with the fall of hemolytic signs. When corticosteroids were reduced, hemo-globin level rose to 12 g/dL, although laboratory evidence of hemolysis persisted. A second course of rituximab, with the same dosage schedule, was administered. To date, four months after last rituximab infusion, the patient is free of treatment, hemoglobin level is 14.5 g/dL and no hemolysis relapse has been observed and the B-cell clonal population no longer detectable even by a nested tailored PCR. Warm and cold autoimmune hemolytic anemia usually benefits of corticosteroids therapy, but long period of maintenance treatment are required, side effects are common and relapse often occurs at withdrawal of prednisone. The first course of rituximab achieved the almost complete restoration of normal hemoglobin level, while the second one led to the complete remission of the hemolytic disease. To our knowledge rituximab has so far efficacy only in CAD associated with an overt lymphoproliferative disease. This is the first reported case of mixed warm and cold autoimmune hemolytic anemia not associated with lymphoma, which has successfull responded to rituximab therapy.
The β-thalassemic gene determines the severity of the clinical picture: d, e, f groups resemble a thalassemia trait, while b and c are indistinguishable from Cooley's anemia. HbLep homozygotes have an intermediate severity. The association between HbLep and Hbs (g group) reduces the seriousness of drepanocytic syndrome. Only one patient has died at age of 31 years for causes not related to thalassemia (severe psychosocial impairment leading to treatment refusal). The heart failure incidence is very low (6%).

The most frequent endocrinopathy is hypothyroidism. Seventy-six percent of patients are positive at HCV antibodies; among these, one-half have chronic hepatitis. Our data are substantially in keeping with the literature; nevertheless the large number of cases and the long follow up provide further insights about the clinical course and prognosis of this hemoglobinopathy.

**PO080**

**TREATMENT WITH CYCLOSPORIN-A IN REFRACTORY AUTOIMMUNE HEMOLYTIC AND IMMUNE THROMBOCYTOPENIC PURPURA**

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Aim of the work. To evaluate the efficacy of cyclosporin A (CsA) in the therapy of refractory autoimmune hemolytic anemia (AHA) and idiopathic thrombocytopenic purpura (ITP). Patients and Methods. Eleven patients with resistant autoimmune cytopenia were treated with CsA. Five patients had AHA, five had ITP and the last one had an Evans syndrome. Two patients developed AHA during B-chronic lymphocytic leukaemia and Hodgkins lymphoma. One patient had thrombocytopenia secondary to systemic lupus erythematosus (SLE) All pts were refractory to corticosteroids and at least to one more treatment such as, pulse high-dose methyl-prednisolone, cyclophosphamide, splenectomy, vincristine, azathioprine and gammaglobulins. The ratio M/F was 6/5. The median age was 58 year (range 14-86). CsA was started at the dose of 2.5 mg/kg twice a day, and then adjusted to maintain therapeutic serum levels of 60-240 ng/mL. Prednisone 0.25-1 mg/kg was continued as long as a stable hematological response was obtained. CSA therapy was introduced from 1 to 54 months from diagnosis (median 20 months) and was given for a median time of 11 months (range 2-18). The median follow-up time from starting CSA therapy was 17 months (range 11-71). Results. a) All 5 pts with AHA have obtained a complete remission (CR) by two months of therapy and 2 patients became Coombs negative. The first patient stopped therapy after 2 months because of severe Pneumocystis carinii pneumonia and he still remains in CR from 2 years; the second patient refused to go on therapy because of poor compliance and she relapsed one year later; the third patient died because of progression of his lymphoproliferative disease, while he was in CR for AHA; the fourth patient relapsed ongoing CSA therapy, she was rescued adding vincristine and prednisone and she is currently maintaining a Coombs negative CR with low dose of CSA; the fifth patient is off therapy in CR. b) Two out of 5 patients with ITP had a CR. One patient reached a durable CR just one month from starting the therapy. One patient discontinued treatment after 18 months in PR and then spontaneously converted in CR two months later. The patient suffering from SLEx achieved a PR. Two last patients didn't respond and they discontinued therapy 8 weeks later. c) The patient affected by Evans syndrome entered CR but, ongoing CSA therapy, she relapsed when steroid was withdrawn. A second CR was obtained with more gradually prednisone tapering. Side effects: One patient during therapy developed a life-threatening Pneumocystis carinii pneumonia. One patient died of encephalitis, one year after CSA interruption. Both have been long treated with corticosteroids. Moderate hypertension, hypertriasis, gingival hyperplasia was observed in only one case. No one developed renal failure. Conclusions. CSA is a highly effective treatment in steroid-refractory AHA and ITP. In our experience eight out of eleven patients showed a CR and one a good PR. Responses have been prompt and sustained in all patients with AHA, but more unpredictable in patients with ITP. Infective disease prophylaxis must be considered, mainly in patients treated for long time with steroids.

**PO082**

**ENDOTHELIAL ACTIVATION MOLECULES IN β-THALASSEMIC PATIENTS**

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β-thalassemias (β-Th) represent a heterogeneous group of congenital anemias characterized by reduced or absent β-globin chain synthesis leading to ineffective erythropoiesis, chronic hypoxia and iron overload, this last representing one of the major cause of systemic tissue damage occurring in Th subjects. Since endothelial cells take up and store iron, they could likely represent one of the first sites of iron-induced oxidative injuries, contributing significantly to systemic iron-related organ damage. Latent endothelial cell damage may be indirectly revealed by the presence of circulating soluble molecules, such as vascu-
lar endothelial growth factor (VEGF), vascular cell adhesion molecule-1 (s-VCAM-1) and intercellular cell adhesion molecule-1 (s-ICAM-1). In this report, serum levels of the above endothelial cell markers, together with serum erythropoietin values, have been evaluated by ELISA technique in 34 B-Th patients (18 B-Th major, 16 B-Th-intermedia; 12 males and 22 female, mean age: 34 yrs), and 10 healthy control subjects, sex- and age-matched. All patients, who underwent regular transfusion regimens (in order to maintain Hb levels > 9 g/dL) and iron chelation therapy, were studied at least 15 days before transfusion.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Controls</th>
<th>B-Th</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/mL)</td>
<td>154±149.29</td>
<td>437.97±269.21</td>
<td>0.05</td>
</tr>
<tr>
<td>s-VCAM-1 (ng/mL)</td>
<td>1533.1±225.7</td>
<td>1892.3±522.93</td>
<td>0.3</td>
</tr>
<tr>
<td>s-ICAM-1 (ng/mL)</td>
<td>599.1±140.99</td>
<td>838±72.36</td>
<td>0.01</td>
</tr>
<tr>
<td>Epo (mIU/mL)</td>
<td>7±3</td>
<td>78±50.49</td>
<td>0.003</td>
</tr>
</tbody>
</table>

As compared to normal controls, all patients showed a significant increase in serum levels of all the above endothelium activation markers, which did not correlate with either Hb or Epo levels. No significant difference of these parameters was found between major and intermediate Th subjects, when evaluated separately. Whatever the cause of these findings (iron-dependent endothelial injury, vascular transfusion-related stress or chronic hypoxia), the high levels of endothelial cell markers in Th patients represent a sign of chronic endothelial cell activation, which in turn could play a significant role in either the activation of the coagulative system or the systemic tissue damage which characterizes the thalassemic syndromes.

PO004
IRON STATUS AND HFE GENOTYPE IN ERYTHROCYTE PYRUVATE KINASE DEFICIENCY: STUDY OF ITALIAN CASES

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Information on iron status in inherited disorders of red cell metabolism is so far scanty. Increased serum ferritin (SF) levels have been reported in PK deficiency and related to the concomitance of different factors such as hemolysis, splenectomy, and heterozygosity for hereditary hemochromatosis (HH). The hemochromatosis gene (HFE) has not, however, been extensively investigated in this disease. We evaluated the iron status and searched for mutations C282Y and H63D in the hereditary hemochromatosis gene (HFE) in 34 pyruvate kinase (PK) deficient patients (14 M and 20 F; median age 19, range 0-43) from 29 unrelated families. Nine had received multiple transfusions, of whom 6 were splenectomized and 8 underwent chelation therapy. Of the 25 never transfused patients, 4 were splenectomized and 2 (one splenectomized) had needed repeated cycles of iron chelation. None of them had evidence of other diseases known to raise SF concentration. Thirteen of the 25 non-transfused patients displayed increased serum ferritin concentration, in the absence of conditions known to raise this parameter. HFE genotype was abnormal in 9 out of 34 patients. The allele frequency was 1.8% for mutation C282Y and 16.1% for H63D. Non-transfused subjects with abnormal genotype had serum ferritin and transferrin saturation values significantly higher than those with wild-type genotype. Of the twelve, adult non-transfused patients with increased iron status parameters, one was C282Y homozygous, one compound heterozygous for C282Y and H63D, 3 H63D heterozygous, and 7 had a normal HFE genotype. Serum ferritin and transferrin saturation were not related to hemoglobin, reticulocytes and bilirubin concentration. At multivariate analysis serum ferritin was independently associated with age and gender, but not with splenectomy and HFE genotypes. The evaluation of the iron status profile of 10 patients (3 with abnormal and 7 with wild-type HFE genotype) with at least ten years follow-up showed that overt iron accumulation requiring iron chelation occurred only in the three patients (two of whom splenectomized) with mutated HFE gene. HFE genotyping may therefore be useful to identify subjects at higher risk of iron overload and to prevent clinical disease complications.
Increased erythroid apoptosis is a specific pathophysiologic lesion in β thalassemia intermedia in which the proportion of erythroid precursors undergoing apoptosis has been reported to be 3- to 4-fold greater than in controls. It has been described that several members of Bcl-2 family proteins constitute a critical intracellular check-point, within a common cell-death pathway, that determines a cell susceptibility to apoptosis. This decisional set point is governed by the pro-apoptotic (Bax/Bad/Bcl-xS) versus anti-apoptotic (Bcl-2/Bcl-xL) Bcl related proteins ratio. In this study we assessed by competitive quantitative PCR analysis, Bcl-2 Family mRNA levels in circulating erythroblasts of 15 patients with β-thalassemia intermedia to evaluate in vivo the role of Bcl-2 genes on thalassemic erythoblast survival. Absolute quantitation (expressed as molecules/micrograms of total mRNA) was accomplished with the use of standard curve. Here we present the evidence that apoptosis inhibitory product Bcl-xL was regularly expressed at higher intensity than Bcl-xS or Bad and Bax in all the patients while Bcl-2 mRNA was not detected in any samples. A similar expression pattern of Bcl-2 genes family has been observed on umbilical cord blood derived erythroblasts from physiological pregnancies, used as normal controls. Quantitative PCR analysis showed highly variable levels of both anti-apoptotic (Bcl-xL) and pro-apoptotic (Bax + Bad + Bcl-xS) mRNAs; the Bcl-xL levels averaged 3.6 E15 (range 1.5×–1.87×) versus 5.8× (range 5.5×–5.7×) of pro-apoptotic mRNAs. The ratio between anti-apoptotic versus pro-apoptotic mRNAs was 6.2 E5 (range 2.7×–6.1×), suggesting a highest prevalence of erythroid cell survival signals on thalassemic circulating erythroblasts. No statistical correlation was observed between the mRNA levels of Bcl-2 gene Family and thalassemic genotype, clinical thalassemic findings (degree of anemia, transfusion therapy, splenectomy) or total globin chain imbalance. Finally the mean level of anti-apoptotic versus pro-apoptotic mRNAs on thalassemic circulating erythroblasts was about 3000 fold greater than that found on the erythroblasts derived from umbilical cord blood (ratio 6.2× and 2.2× respectively). This expression pattern of prevalent inhibitory product is unexpected in circulating thalassemic erythroblasts, which are characterized by presence of globin chain imbalance at all stage of red cell maturation, nevertheless they are in agreement with our previous study which showed a low mean value (2.7%) of positive annexin V circulating erythroblasts precursors. In conclusion, it might be that these erythroid cells escape programmed cell death by high expression levels of Bcl-xL gene which preserve them from marrow and peripheral macrophage phagocytosis. These data suggest that Bcl-xL gene might be a good candidate involved in the anti-apoptotic pathway which preserves erythroid precursors from programmed cell death of thalassemic erythropoiesis.

(Financed in part by the Ricerca Sanitaria Finalizzata RAS)

PO087
LATE OCCURRENCE OF CD3+ LARGE GRANULAR LYMPHOCYTE LEUKEMIA WITH PURE RED CELL APLASIA IN A RENAL TRANSPLANT PATIENT SUCCESSFULLY TREATED WITH ORAL METHOTREXATE

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Post transplant lymphoproliferative disorders (PTLDs) may occur after solid organ and bone marrow transplantation. More than 90% of PTLDs are associated with Epstein-Barr virus infection and are of B-cell lineage. T-cell lymphoproliferative disorders are uncommon, and among them, large granular lymphocyte (LGL) leukemias are exceedingly rare. We report the late occurrence of a T-LGL associated with pure red cell aplasia (PRCA) in a renal transplant patient responsive to oral methotrexate treatment. A 35-year old man was referred to our department with severe normocytic normochromic anemia in June 2000. He received a cadaveric renal allograft 13 years earlier because of end-stage renal disease. Immunosuppressive therapy consisted of cyclosporin A (CyA) at 4 mg/kg/day and methylprednisolone at 0.1 mg/kg/day on alternative days. In January 1998, he was first noted to have absolute lymphocytosis (55% to 71% lymphocytes) with LGL morphology, expressing CD3, CD8, CD16, CD57 and lacking CD56. Physical examination was unremarkable; the abdominal ultrasoundography revealed mild splenomegaly without lymphadenopathy. Four months before admission haemoglobin dropped to 6 g/dL with persistent gamma-delta T-cell lymphocytosis (34%) and he was given red blood cell transfusions. At the time of admission, laboratory findings were as follows: hemoglobin 6.7 g/dL, platelet count 175 × 10^9/m^3, white cell count 4.4 × 10^9/m^3 with 44% neutrophils, 40% lymphocytes. More than 70% of lymphocytes were of LGL morphology and expressed CD3, CD8, CD57, CD11c, γ-δ-T, CD16, δ-T-Cell receptor (TCR), but not CD56. The molecular tests for viruses such as B19, EBV, CMV and HHV6,7,8 were negative. Bone marrow biopsy showed pure red cell aplasia. The cytogenetic study and Southern blot analysis for the β-TCR gene rearrangement resulted negative. Despite previous CyA treatment, a diagnosis of LGL leukemia with PRCA was made. Treatment with cyclophosphamide (1 mg/kg/day) and methylprednisolone (0.05 mg/kg/day) was instituted but during the following four months, the neutropenia worsened (white cell count dropped from 4.4 × 10^9/m^3 to 1.3 × 10^9/m^3) and the number of transfused packed red cells increased. In September 2000, oral low-dose methotrexate was started at 5 mg weekly with escalation up to 15 mg associated with methylprednisolone (0.05 mg/kg/day). He became transfusion independent within four months. The hemoglobin level is currently maintained between 10.5 g/dL and 11.5 g/dL; mild neutropenia is still present (white cells 2.1 × 10^9/m^3 with 73% of neutrophils). The bone marrow core biopsy now shows a normocellular pattern. We report the fifth case in the literature of LGL occurring after transplant. Despite long term immunosuppressive treatment, autoimmune manifestations such as PRCA may occur, although rarely, in solid organ transplant patients. Methotrexate is apparently a good therapeutic option for patients with LGL/PRCA resistant to other immunosuppressive drugs.

PO088
AUTOMATED ANALYSIS OF BONE MARROW FLUID

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Automated quantitative analysis of cellular composition of bone marrow fluid has always been difficult using hematologic analyzers. A number of problems, in fact, arise from difficulties in erythroblast identification, contamination by fat particles, heterogeneity of cell types and maturation levels and presence of low frequency and very large cells. Methods. The Sysmex XE-2100 automated blood cell counter can effectively count erythroblasts using laser light flow cytometry and DNA fluorochromes. In this study we have evaluated the performance of the Sysmex XE-2100 for analyzing bone marrow cells. We have assessed imprecision of the Sysmex XE-2100 NRBC counting using linear regression and differences between duplicates, comparability, throughout comparison with NRBC count obtained with a microscope reference cell count of 500 cells using the NCCS-H2OA method and clinical usefulness of the Sysmex XE-2100 NRBC method from the observation of instrument reports in patients with hematological disorders. Study samples. All samples were 1-2 mL of bone marrow fluid collected in K2-EDTA, analyzed within 4 hours from venipuncture using the XE-2100. Before aspiration, samples were gently mixed by inversion. No filtration nor any other treatment of samples was carried out. Microscope analysis was carried out on May-Grünewald-Giemsa stained bone marrow smears. A total of 500 cells per film was counted. Since the XE-2100 does not include NRBC count in the WBC count, all results were corrected to a total nucleated cells count (TNCC) calculated as TNCC = NRBC + WBC. Results. Reproducibility of the XE-2100 measurements was assessed through duplicate analysis of 85 samples. Coefficients of variation were below 15%. In 47 out of 147 samples analyzed the instrument could not calculate a complete differential count. We compared the proportion on bone marrow granulocytes and erythroblasts by the XE-2100 with the microscope count using linear regression and analysis of differences on 100 samples, in which a complete computation was available, out of a total of 147 samples. Correlation between XE-2100 and the microscope was excellent for granulocyte percentage (R² = 0.756). Analysis of differences indicated a very good agreement in the great majority of samples, with a mean difference (XE-2100 versus microscope) of -0.9%. Correlation between XE-2100 and the microscope was slightly lower for erythroblast percentage (R² = 0.576), with a mean difference (XE-2100 versus microscope) of -13.4%. There was a tendency of the XE-2100 to slightly underestimate erythroblast percentage in most of the samples. Clinical usefulness. Instrumental total nucleated cell count was a good indicator of marrow cellularity. The observation of instrumental cell distribution plots provided the morphologist with an immediate general perception of the overall quality and characteristics of the samples in leukemia, hypoplasia and erythroid hyperplasia.
β-thalassemia syndromes are the most common hereditary chronic hemolytic anemia due to impaired globin chain synthesis. The imbalance between α and β chains plays a crucial role in producing oxidative stress. A low molecular weight iron fraction not bound to transferrin (NTBI) is always detected in sera of these iron overloaded patients and such iron is known to generate oxygen free radicals followed by peroxidative tissue injuries. Malondialdehyde (MDA), a terminal compound of lipid peroxidation, is widely used as an index of the oxidative status. Non-enzymatic antioxidants, such as albumin, GSH, ascorbic acid, vitamin E, bilirubin and uric acid, are important parameters to assess antioxidant status in thalassemia and in other pathological conditions associated with secondary iron overload. In this study, using a gas chromatography-mass spectrometry (GC-MS) method, both free and total MDA were measured in serum of 21 β-thalassemia major (TM) patients and of 13 β-thalassemia intermedia (TI) patients. Controls were 10 healthy members of the laboratory staff. Moreover the MDA levels were related to the antioxidant capability to counteract free radical formation, taking into account the iron overload. NTBI was measured in serum by HPLC in the presence of CP22 chromogen after nitritetraacetic acid chelation and ultrafiltration. Lipid peroxidation was evaluated as serum free total malondialdehyde (MDA) measured by selected ion monitoring gas chromatography-mass spectrometry. Total radical-trapping antioxidant parameter (TRAP) was tested in serum by fluorescent monitoring of trolox-induced lag phase using the free radical probe dichlorofluorescein-diacetate (DCFH-DA). Iron status levels of erythroid hyperplasia were defined. In this report we correlate some clinical features to the level of erythroid hyperplasia. Seventy-eight consecutive patients with severe β-thalassemia were studied. The results are summarized in the Table.

Clinical features

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Erythroid hyperplasia</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15±5</td>
<td>13±7</td>
<td>10±7</td>
</tr>
<tr>
<td>β^+β^- (n° pts)</td>
<td>16±9</td>
<td>12±19</td>
<td>11±16</td>
</tr>
<tr>
<td>PRB units (n°)</td>
<td>196±126</td>
<td>162±32</td>
<td>105±93</td>
</tr>
<tr>
<td>Hepatic iron concentration (mg/gm)</td>
<td>20±100</td>
<td>12±7</td>
<td>10±7</td>
</tr>
<tr>
<td>Chronic hepatitis (A or B)</td>
<td>15±5</td>
<td>9±22</td>
<td>9±18</td>
</tr>
</tbody>
</table>

Results statistically different: *L vs M; #L vs H; &M vs H.

The definition of a quantitative method to measure the extent of the erythroid expansion could be a useful addition to our current clinical assessment to define the risk of graft failure before bone marrow transplantation for severe β-thalassemia. This work was supported by the Berloni Foundation against Thalassemia.

P0091
CLINICAL EVALUATION OF A NEW AUTOMATED BLOOD CELL COUNTER WITH RETICULOCYTE ANALYSIS

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Automated reticulocyte counting has increased precision and accuracy of this assay compared with the traditional manual count supravital staining procedure. The reticulocyte measurements, including immature reticulocyte fraction (IRF) and mean reticulocyte volume (MRV), are a fundamental indicator of the functional state of erythropoiesis in several conditions such as in the differential diagnosis of anemias, in the follow-up of patients treated with iron, folate, vitamin B12 or recombinant human erythropoietin, as well as for evaluating engraftment after bone marrow transplantation or recovery after chemotherapy. Automated reticulocyte counting methods are based on...
We report a case of β-thalassemia intermedia (BT) in a patient with secondary pulmonary hypertension (PHT). The patient is a 33-year old male who was diagnosed with BT at the age 4. He was splenectomized at age 18 and was able to maintain hemoglobin (Hb) levels ranging from 8.5–9.5 g/dL without transfusion therapy for the first 31 years of life. During the last 3 years, his Hb levels had progressively decreased (Hb<7.5 g/dL). Iron chelation therapy was started at the age 32 followed by regular transfusion. Patient suffered fatigue and was treated with anticoagulant for chronic atrial fibrillation. Recent echocardiography revealed right ventricular enlargement and a mild-moderate tricuspid valve regurgitation due to intolerable side effects. Sildenafil is a selective and potent inhibitor of phosphodiesterase type 5 (PDE5), which hydrolyses cGMP and promotes smooth muscle relaxation in lung vasculature. Sildenafil was given at 50 mg/day for 1 month and then increased to 50 mg 3 × day. Echocardiography performed 3 months later showed a stable or slightly decreased PAP (max 54 mm Hg) whereas respiratory function tests indicated an improvement in the restrictive ventilation. There was no significant decrease in systemic artery pressure and the patient reported an improvement in general health. In vitro study has shown that another PDE inhibitor, zaprinast, increases γ-globin chain production via soluble guanylate cyclase-cGMP-dependent protein kinase pathway. Sildenafil is an attractive alternative to conventional therapy for PHT. Whether sildenafil has any effect on γ-globin synthesis in vivo is of particular interest to BT patients with secondary PHT and should be further investigated.

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The goals of transfusion therapy in the thalassemia syndromes are twofold: 1) to prevent anemia, 2) to suppress endogenous erythropoiesis that leads to many of the disabling complications associated with thalassemia. A pretransfusion nucleated red blood cell (NRBC) count less than 5 per 100 white blood cells is considered to be a useful index of erythroid marrow suppression. The XE-2100™ is the latest hematology analyzer to be introduced to the market and utilizes the technology of fluorescence flow cytometry to quantitate the NRBC. This automated count is more accurate and precise than the manual method due to the large number of cells counted and the lower limit of detection. The Authors evaluated the NRBC counts in twelve transfusion-dependent thalassemia patients at different levels of pretransfusion hemoglobin (Hb). The results show: 1) for every patient the increase of Hb level is associated with the decrease of NRBC, as expected; 2) the suppression of ineffective erythropoiesis, as determined by the disappearance of NRBC in the peripheral blood, is achieved for each patient at different hemoglobin concentration. The table data shows: 1) a 4-month old patient (Z.G.) with Cooley’s anemia, whose ineffective erythropoiesis was almost completely suppressed at Hb level of 8.3 g/dL. The XE-2100™ is effective in HCL, the well-known capacity of interferon to promote autoimmunity makes its use controversial in the presence of HCL-related autoreactive disorders. The potential therapeutic role of the drug in such occurrences is largely unknown. A 57-year old female presented in September 1998 with weight loss, fatigue and jaundice. Hepatosplenomegaly was found. Peripher-
al blood showed hemoglobin (Hb) 68 g/L, white blood cells 4.8 × 10^11/L (neutrophils 51%, lymphocytes 33%, monocytes 16%), platelets 153 × 10^11/L, MCV 104 fl, and aniso-poikilocytosis with microspherocytes. Reticulocytes were 660 × 10^3/μL. Lactate dehydrogenase, total unconjugated bilirubin and β2-microglobulin were increased to 537 (range 10-500) U/L, 2.45 (range 0.25-1 and 0.2-0.7) mg/dL and 3.1 (range 0.6-2.6) microg/mL, respectively. Haptoglobin was 8 (range 32-205) mg/dL. The Coombs' test was positive for IgG and C3d. Circulating immune complexes were unremarkable. Hepatitis B surface antigen (HbsAg) was positive. Hepatitis B virus (HBV)-DNA was undetectable by polymerase chain reaction. Liver function tests suggested an HbsAg carrier status. Bone marrow showed hypercellularity, erythroid hyperplasia, and no lymphoid infiltration. An idiopathic AHA was diagnosed. Oral prednisone 1 mg/kg/day was started with Hb and haptoglobin normalization, and then tapered at a rate of 10 mg per week, conditioning HBV-DNA positivization and periodical hepatitis reactivations. After 7 months, chronic compensated hemolysis recurred. Maintenance prednisone doses were continued. Beta2-microglobulin progressively increased. In July 1999, pancytopenia and further spleen enlargement developed. Peripheral hairy cells phenotypically staining for CD19, CD20, CD22, CD11c and CD25 appeared. Marrow and liver biopsies showed focal infiltration with typical hairy cells, and liver cirrhosis. HCL was diagnosed. In January 2000, interferon-α 3 MU s.c. thrice p.w. was started. Maintenance prednisone was continued. Shortly after interferon-α initiation, Hb progressively increased and haptoglobin stabilly returned within normal. Peripheral hairy cells disappeared within 4 months, β2-microglobulin and spleen size progressively diminished. Since HBV-DNA positivity persisted, in June 2000 lamivudine 100 mg/day p.o. was started. Interferon-α was temporarily withdrawn until HBV-DNA negativization, which occurred within 1 month and allowed prednisone withdrawal. During interferon-α discontinuation, AHA transiently recurred. Nine months after interferon-α initiation, a partial remission of the HCL was associated with undetectable hemolysis. This case (i) confirms previous observations indicating that immunosuppression is not the most appropriate treatment of HCL-related autoimmune hemolysis (ii) suggests that, although interferon-α is known to promote rather than to cure autoimmunity, AHA recovery may parallel the clinical response of HCL to interferon-α, (iii) indicates that other drugs known to be curative for the HCL could be used successfully on similar occasions.

PO098
AUTOMATIC DIAGNOSIS OF CRYOAGGLUTININEMIA

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Pneumonia caused by mycoplasma pn. is not frequent, and may cause therapeutic problems, mainly among young patients, as it is resistant to broad-spectrum antibiotics. We have recently observed three children with a common story of sore throat, cough, headache, lung opacity to the X-ray, and ampicillin resistance. Basal work-up was normal with the exception of increased ESR and serum IgM, and abnormalities of peripheral blood examination by automatic counter consisting of: reduction of the equivalent Ht=Hb×3, increased RDW, MCH, MCV, RDW % HYPO-E, %MICR-E and HDW. The system software was properly set to automatically calculate also DIBTT1 and DIBTT2 obtained using the following formulas:

\[
\text{DIBTT1}= [(\text{RDW} \times \text{MCV} \times \% \text{HYPO-E}) / (100 \times \text{RBC} \times \% \text{MICR-E}) \times \text{MCH}]
\]

\[
\text{DIBTT2}= [(\text{RDW} \times \text{MCV} \times \% \text{HYPO-E} \times \text{HDW}) \times (\text{Ht} - 3 \times \text{Hb}) / (100 \times \text{RBC} \times \% \text{MICR-E} \times \text{CHCM})]
\]

Hemoglobin fractions were measured by a high performance liquid chromatography system (VHTS, Biorad Laboratories, Segrate, Italy). Statistical processing was performed using the Excel software (Excel 97, Microsoft Corporation, USA). Among the 937 microcytic subjects, BTT was consistently diagnosed by the DIBTT1 and DIBTT2 indexes, provided that the subjects were divided into two groups. DIBTT1 correctly identified all patients whose hemoglobin distribution width (HDW) was between 2.2 and 3.5 g/dL, while DIBTT2 correctly identified all patients with HDW < 2.2 or > 3.5 g/dL. Compared to a detection method based on haemoglobin chromatography, the two new indexes allowed a correct diagnosis of BTT in 84.3% of cases; the concordance was absolute (100%) when a few categories of subjects were excluded (pregnant women, children younger than 7 years, neonatal patients, transfused or hemorrhagic patients, anemic patients given iron treatment and patients in dialysis). Since DIBTT1 and 2 can be directly calculated by the automation in real time at no additional cost, they are excellent candidates to screen healthy adult subjects for BTT.

PO097
TWO NEW HEMOGLOBIN DISTRIBUTION WITH-BASED INDEXES TO IDENTIFY β-TALASSAEMIA TRAIT

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Since the early seventies, a number of indexes have been proposed to detect subjects carrying heterozygous β-thalassemia genes (BTT = β-thalassemia trait), by simple and inexpensive screening methods. We have developed two new indexes, termed discriminating β-thalassemia trait index one and two (DIBTT1 and DIBTT2), which reliably recognize BTT subjects in real time by automatic blood cell counting. Peripheral blood samples from 937 microcytic (MCV <80fL) subjects were evaluated by an automated hematologic analyzer (H3 Bayer), which measures simultaneously the following parameters: RBC, Hb, MCV, Ht; MCH; CHCM; RDW; % HYPO-E; %MICR-E; and HDW. The system software was properly set to automatically calculate also DIBTT1 and DIBTT2 obtained using the following formulas:

\[
\text{DIBTT1}= [(\text{RDW} \times \text{MCV} \times \% \text{HYPO-E}) / (100 \times \text{RBC} \times \% \text{MICR-E}) \times \text{MCH}]
\]

\[
\text{DIBTT2}= [(\text{RDW} \times \text{MCV} \times \% \text{HYPO-E} \times \text{HDW}) \times (\text{Ht} - 3 \times \text{Hb}) / (100 \times \text{RBC} \times \% \text{MICR-E} \times \text{CHCM})]
\]
these cases. In particular, we wish to underline the imbalance of the equivalence (HT=Hb×3), which is reduced (HT<Hb×3) due to reduced count of agglutinated erythrocytes, the increased RDW, due to the presence of double erythrocyte population, the overvaluation of MCV, due to the hemagglutination, (MCV= mean corpuscular volume calculated as follows: (Hb×100)/(CHCM×RBC). MCHC= directly measured by the autoanalyzer. MCHC= (Hb×100)/(MCV×RBC). CHCM = directly measured.

PO099
HETEROZYGOUS β-THALASSEMA AND HOMOZYGOUS H63D HEMOCHROMATOSIS IN A CHILD: A 16-YEAR FOLLOW-UP
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Children with β-thalassemia trait (β-th) present slight ineffective erythropoiesis and erythroid hyperplasia; there is little evidence of moderate increase of iron absorption. Nevertheless, they often suffer from iron deficiency, as do non-affected children, due to the most common causes of iron depletion found during development. In a few patients biological parameters are indicative of increased iron stores. It is probably that other factors are involved in these rare cases. Hereditary hemochromatosis (HFE) is an autosomal recessive disorder that affects 1:200-1:400 individuals of European descent. Most of them are homozygous for C282Y mutation; a few are homozygous for H63D HFE. The association of the β-thalassemia gene and HFE gene is rather rare also in Italy. The relationship between β-thal and hemochromatosis in determining iron overload is controversial. It has been demonstrated that the coinheritance of β-thal and a homozygous C282Y HFE may increase the risk of developing iron overload. Data regarding the association of β-thal associated with mild HFE genotype H63D homozygous, as observed for C282Y homozygous.

PO090
SERUM ERTHROPOIETIN LEVELS IN MEGALOBLASTIC ANEMIA
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We have evaluated serum erythropoietin levels (S-EPO) using commercial radioimmunoassays (DSL-1100) in 27 patients (15 males, 12 females), mean age 69 (range 38-90), with megaloblastic anemia prior to any therapy (25 vitamin B12 deficiency, 2 folic acid deficiency) without liver disease or kidney failure. Reference subjects are 30 healthy persons (S-EPO 5.9±6.4mU/mL; Ht% 42.8±2.7). On the basis of values of S-EPO (72±77mU/mL, range 8-396) and hematocrit (Hct% 22±4.5) we have calculated O/P ratio (0.66±0.14) that showed an inadequate erythropoietin response to anemia: only 6 out of 27 patients have a normal O/P ratio (≥0.80). We have also evaluated LDH (2117±1941U/L) and reticulocyte count using flow cytometry (Ret. N° 329±204 106 µL). S-EPO is inversely related, though non significantly, with hemoglobin (Hb) levels (r=0.256); Hb is inversely and significantly related with serum iron (r=-0.7). Mean values of E/G ratio are higher than normal (1,3±0.7; range 0.5-2.9); only 4 patients have E/G ratio<0.7. We have not found any relation between E/G ratio and S-EPO (r=0.09) nor between E/G ratio and O/P ratio (0.11). In megaloblastic anemia there is an inadequate erythropoietin response; we have not found a well defined correlation between S-EPO and red blood cell precursor mass as measured by bone marrow morphology.
**PO01**

**ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM NORMAL BONE MARROW**

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Mesenchymal stem cells (MSC) are a population of multipotent cells which can be isolated from the adult bone marrow (BM) and retain the capacity to proliferate and differentiate into multiple mesodermal tissues including bone, cartilage, tendon, muscle and fat. We previously reported that the monoclonal antibody (MoAb) to the low-affinity nerve growth factor receptor (anti-NGF-R) stains BM mesenchymal cells (Cattoretti et al., Blood 81:1726, 1993). We now show that LNGF-R antibodies label MSC with high specificity and purity in the adult bone marrow and compare these cells to those isolated by plastic adherence (PA) and CD45-/anti-glycophorin A- selection. Low-density mononuclear cells (LDMNCs) from normal BM were immunomagnetically separated (MACS, Miltenyi Biotech, Germany) with a-NGF-R MoAbs, with α-CD45/-anti-glycophorin A- MoAbs or by means of PA (Pittenger et al.) and grown in IMDM + 20% FBS + FGF (10 ng/mL) to induce fibroblastic differentiation: the proliferation capacity of NGF-R+ cells was always greater than that of CD45-/α-glycophorin A- and PA cells, with expansion values up to 1.5×10^6-fold after 2 months of culture, 2 to 3 log better when compared to the other fractions. Immunophenotypic analysis performed after 2 and 4 weeks of culture always showed a rapid decrease in the expression of NGF receptor, while the great majority of the cells had fibroblastic markers, with a persistence of residual CD14+/CD45+ hematopoietic cells in MSc obtained by plastic adherence even after numerous rounds of selection. To evaluate the clonogenic potential of MSC, CFU-F were assayed in limiting dilution after numerous rounds of selection. To assess the clonogenic potential of these cells, adipogenic and osteogenic differentiation assays were performed. As revealed by Oil Red O staining, NGF-R+ cells showed an increasing number of adipocyte colonies starting from two weeks after seeding, CD45-/glycophorin A- cells gave only a few colonies or single adipocytes and PA cells only differentiated in single adipocytes. Furthermore, NGF-R+ cells driven to osteogenic differentiation showed a higher number of aggregates with an increased expression of alkaline phosphatase activity and calcium accumulation, as revealed by Alizarin Red staining. All these data suggest that positive selection with the low affinity NGF-R antibody is the method of choice to obtain pure and multipotent MSC.

**PO02**

**HUMAN BLADDER CARCINOMA CELL LINE 5637 ACTIVATES INFLAMMATORY CELLS TO PRODUCE HEPARIN-BINDING ENDOTHELIAL GROWTH FACTOR: A POSSIBLE REGULATORY LOOP INVOLVING CXCR4/CXCL12 SYSTEM**

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Heparin-binding EGF-like growth factor (HB-EGF) is a widely expressed EGF superfamily member which binds to EGF receptor tyrosine kinases 1 or 4 (HER-1, -4), supports mitogenic and angiogenic activities for numerous types of carcinoma also inducing secondary factors, such as VEGF, and is the receptor for diphtheria toxin (DT). Using molecular (RT-PCR cloning, Northern blot, flow cytometry, ELISA) and functional (mitogenic activity on BALB/c 3T3 cells, sensitivity to the pro-apoptotic effect of DT) approaches we have previously shown that recombinant GM-CSF can specifically regulate the production of HB-EGF in normal and neoplastic myeloid cells in vitro. We studied now whether cancer cells releasing GM-CSF were able to induce neutrophils (PMN), monocytes (Mo) and lymphocytes (PBL), to produce HB-EGF. We found that the conditioned medium of the human bladder carcinoma cell line 5637, which is rich in GM-CSF, was a powerful inducer of HB-EGF in PMN, Mo and PBL in a fashion similar to that observed in our previous experiments using recombinant GM-CSF in vitro. Tests with neutralizing anti-GM-CSF and anti-GM-CSF receptor mAbs specifically inhibited the production of HB-EGF by PMN, Mo and PBL. The cell line 5637 was able, via GM-CSF, to induce potentially infiltrating reactive PMN, Mo and PBL to produce HB-EGF which would in turn support the proliferation of the carcinoma cells themselves, which express HER-1 tyrosine kinase. Though HB-EGF is an autocrine growth factor for some types of epithelial cancers, our data provide evidence that inflammation- and immunity-related cells can be driven by the neoplastic cells which they infiltrate in vivo, to produce factors, namely HB-EGF, favoring neoplastic growth and angiogenesis. Interestingly, both inflammatory cells and cancer cells express CXCR4 chemokine receptor and co-localize to tissues rich in CXCL12 (formerly SDF-1, the ligand for CXCR4). Preliminary data show that stimulation of CXCR4 via CXCL12, induces inflammatory cells to release cytokines, favoring both GM-CSF and HB-EGF up-regulation. Our data provide evidence that chemokines relevant to cancer and inflammatory cell homing can also trigger the production of factors involved in angiogenesis and cancer growth.

**PO03**

**A NEW HUMAN STEM CELL LINE EXPRESSING CD33 IN THE ABSENCE OF SURFACE CD34 ANTIGEN**

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Recent evidence indicates that the most primitive blood hematopoietic stem cell (HSC) resides among cells lacking CD34
and lineage commitment markers (CD34, Lin). Unfortunately, these primitive HSC are extremely rare (0.2%) among the CD34+ cell population and their identification has been hampered by the laborious isolation procedure and the small yields of cells obtained by various methods of isolation used so far. Therefore a priority has been to search for cell lines with HSC characteristics in order to facilitate research in this area. We have established a human fibroblast-like cell line, designated GM-490, from the marrow of a patient with acute lymphoblastic leukemia. GM-490 cells have the morphology typical of adherent fibroblasts, cytogenetic studies revealed a complex 56xy karyotype and molecular analysis studies excluded their leukemic origin. GM-490 cells express CD29, the β1 integrin receptor; CD36, the thrombospondin receptor; CD71, the transferring receptor but lack CD34, HLA-DR and all lymphoid, erythroid and myeloid markers. We have found that the majority of GM-490 cells express the novel hematopoietic antigen CD133 and the SCF receptor, c-kit, (CD117). Large amounts of SCF (3711pg/mL) are secreted in the supernatant, suggesting an autocrine mechanism of proliferation. We suppose that GM-490 arose from the marrow microenvironment where the majority of CD34- HSC reside in a quiescent state. This cell line represents a useful model to study the designated hierarchy of HSC.

POD03
A HIV-1 POL GENE DERIVED SEQUENCE INCREASES THE EFFICIENCY OF TRANSDUCTION OF HUMAN NON DIVIDING MONOCYTES AND T-LYMPHOCYTES BY LENTIVIRAL VECTORS

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Gene therapy would greatly benefit from the development of viral vectors capable of transducing resting cells. We have investigated the capacity of two HIV-1- derived lentivectors, differing for the presence of a 118 bp pol fragment, to infect either cell lines or primary cells of different hematopoietic lineages. We show that both vectors work well achieving higher frequency of transduction compared to a Moloney- derived oncovector. Infection of proliferating human T-lymphocytes revealed that the lentivirus carrying the pol fragment (cPPT) is the most effective, transducing up to 78% of cells compared to 42% for that without the additional sequence (no cPPT). In contrast, when tested on proliferating tonsillar B-cells, neither of the lentiviruses showed any transducing activity, in spite of very efficient transduction of all studied B-cell lines. Most interestingly, the two viruses differed in their capacity to infect non- proliferating primary cells: only the cPPT virus showed significant transduction (up to 29 %) of T-lymphocytes and this only after IL-2 activation of the cells, whereas both lentiviruses showed high efficiency on monocytes, with the cPPT virus transducing 72-90% of the cells with high expression levels. Finally, neither virus transduced resting B lymphocytes at all. These data show that the pol sequence significantly improves the vectors with respect to non- proliferating T-lymphocytes and monocytes, although fully resting T-cells, in the absence of IL-2, are not efficiently transduced by any vector. Finally, primary B-cells, but not B-cell lines, are completely resistant to infection by lentiviruses.

POD04
IN VITRO GENERATION OF MUSCLE CELLS FROM ADULT HUMAN BONE MARROW

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Bone marrow, the major source of adult hematopoietic stem cells (HSCs) also contains mesenchymal stem cells (MSCs) which have the ability to proliferate and differentiate into multiple cell types contributing to the regeneration of mesenchymal tissues such as bone, cartilage, adipose and stroma. Some investigators have reported sporadic in vitro formation of myotubes from bone marrow mesenchymal stem cells using 5- azacytidine (Wakitani, 1995) or amphotericinB (Phinney, 1999). We now report the generation of muscle cells from unfractioned human bone marrow in culture. In particular, bone marrow cells obtained from ribs resected at the time of thoracotomy in 7 lung cancer patients were plated in DMEM supplemented with 10% FCS and the supernatant of the cultures replated weekly. The adherent fractions and the supernatant of the cultures were analyzed by immunohistochemistry, Western blot and RT-PCR. A variable percentage of desmin+ cells (ranging from 5 to 30-40%) was observed in the adherent fractions mixed with a majority of TE7+ and CD14+ cells. Starting from the third and the fourth adherent fractions, numerous long multinucleated desmin+ cells (myotubes) were observed in 4 cases. These data show, for the first time, that myotubes can be generated from the adult human bone marrow. In view of the recent reports of the in vivo regeneration of both skeletal and cardiac damaged muscle after bone marrow transplant in mice, these results could open the way to cell therapies for muscle regeneration and repair.

POD05
DEGRADATION OF THE CD3-ζ CHAIN OF T ACUTE LYMPHOCYTIC LEUKEMIA CELLS BY THE PROTEASOME COMPLEX

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Ubiquitination and the consequent degradation of ubiquitinated proteins by the proteasome pathway plays an important
role in the control of several cellular processes, including cell cycle progression, signal transduction, transcriptional regulation and apoptosis. Abnormalities of this process have been implicated in the pathogenesis of malignant transformation. The integrity of the T-cell receptor complex (TCR/CD3) transduction machinery is central to T-cell development and to T-cell effector function. The \( \zeta \) subunit of the T-cell antigen receptor complex is thought to be required for targeting nascent receptor complexes to the cell surface and for receptor-mediated signal transduction. T cells from a variety of hematologic and solid malignancies show a defective TCR \( \zeta \)-chain expression, which is sometimes reversible after T-cell stimulation. In this study we analyzed the TCR-CD3-\( \zeta \)-complex and the signal transduction apparatus of T acute lymphoblastic leukemia (T-ALL) blast cells and verified the possible presence of aberrations of the ubiquitin-proteasome system. Eight out of 8 T-ALL studied showed a marked reduction in the expression of the \( \zeta \)-chain, evaluated by immunoprecipitation and Western blot analysis. A variety of tyrosine kinase proteins, such as p56\( \text{ck} \), ZAP70 and SYK, were normally present. The expression of the FcR,\( \gamma \)-chain (a protein belonging to the \( \gamma \) subfamily) was also evaluated and found absent in all cases. In order to confirm that this aberration was specific to T-ALL blasts, we investigated 2 cases of large granular lymphocyte expansion, a disorder of mature circulating CD3\( ^+ \)CD56\( ^+ \) lymphocytes, and found that cells obtained from these patients expressed the \( \zeta \)-chain at normal levels. The reduction of the \( \zeta \)-chain protein was not reversible after 72 hours’ stimulation with the anti-CD3 mAb (1 \( \mu \)g/mL), IL-2 (1000 U/mL) or the combination of both. To evaluate whether the reduced protein expression corresponded to a defect at the mRNA level, a Northern blot analysis was performed in one case. T-ALL blasts, when compared to T lymphocytes from normal donors, presented a comparable quantity of mRNA encoding for the \( \zeta \)-chain. We therefore hypothesized that the observed reduction of protein expression could be secondary to an increased degradation at the proteasome level. Following incubation of blast cells with epoxomicin, a potent and selective inhibitor of the proteasome, a marked increase of the concentration of the \( \zeta \)-chain was observed in the 4 cases studied. Moreover, an increase in the CD3 protein expression on the cell surface was documented. The results of this study indicate that the expression of the \( \zeta \) subunit of the T-cell antigen receptor complex is consistently reduced in T-ALL and that degradation of the protein is mediated by the proteasome system. Reconstitution of \( \zeta \)-chain expression seems to regulate the maturation of these cells. This abnormality may play a role in the altered differentiation pathway of T-ALL and thus account for the developmental block of these cells and for the uncontrolled expansion of immature T-cell blasts.

**PO108**

**TWO NOVEL MUTATIONS IN THE FERROCHELATASE GENE IN ITALIAN PATIENTS WITH ERYTHROPOIETIC PROTOPORPHYRIA**

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Erythropoietic protoporphyria (EPP) is an autosomal dominant disease due to reduced activity of ferrochelatase (FECH), a mitochondrial enzyme that catalyzes the insertion of iron in the protoporphyrin molecule in the final step of the heme biosynthetic pathway. The disease is biochemically characterized by increased protoporphyrin levels in erythrocytes, plasma and feces. The clinical manifestations have a childhood onset characterized by mild to moderate cutaneous photosensitivity, mild anemia and in 5-10% of the cases by progressive hepatic fail-

**PO107**

**HUMAN RECEPTOR-TYPE TYROSINE PHOSPHATASE \( \gamma \) IS EXPRESSED IN HEMATOLOGIC MALIGNANTIES AND SELECTIVELY INHIBITS ERYTHROID DIFFERENTIATION**

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The initiation, extension and termination of tyrosine phosphorylation is critical for cell homeostasis and is determined by the concerted activities of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Little is known about the physiological roles of PTPs. The PTP superfamily, which is characterized by at least one conserved catalytic domain, includes: classical PTPs comprising transmembrane (receptor-like PTPs) and non-transmembrane (non-TR) forms, dual-specificity (DSPs) and low molecular weight (LMPs) phosphatases. Experimental evidence for a role of PTP\( _\gamma \), a transmembrane component of the PTP superfamily, in hematopoiesis has come from the study of the effects of the perturbation of PTP\( _\gamma \) expression in the differentiating murine embryonic stem cells ES-D3. In appropriate culture conditions these cells differentiate showing a sharp peak of PTP\( _\gamma \) gene expression between 14 and 16 days of their differentiation into the different cell types. In this model, the clones derived from PTP\( _\gamma \)-antisense transfected ES-D3 cells did not produce hematopoietic colonies, while clones derived from PTP\( _\gamma \)-sense transfected ES-D3 cells displayed a block in the erythro-myeloid differentiation program. In the present study we evaluated PTP\( _\gamma \) expression in human hematopoietic cells. PTP\( _\gamma \) expression was confined, in healthy subjects, to the macrophages of the thymus, to the germinal center macrophages of the lymphatic follicles of spleen, lymph nodes and tonsils and to the lymphocytes of the follicular marginal zone of the spleen. Bone marrow and peripheral blood cells did not express PTP\( _\gamma \). The study was then focused on hematopoietic malignancies: 26 leukemia/lymphoma cell lines and 52 cases of acute myeloblastic and lymphoblastic leukemia were analyzed. PTP\( _\gamma \) expression was observed in 50% of the cell lines and 30% of the primary leukemias. To determine whether PTP\( _\gamma \) expression could alter hematopoietic cell differentiation, K-562 leukemic cells, which do not express PTP\( _\gamma \) and can be induced to differentiate toward erythroid or megakaryocytic lineages, were stably transfected with a PTP\( _\gamma \)-cDNA. K-562 erythroid differentiation induced by sodium butyrate was reduced in the transfected clones as compared to the control clones. PTP\( _\gamma \) expression did not affect phorbol esters or staurosporine-induced megakaryocytic differentiation of the same clones. Thus, we identified the cell types that constitutively express PTP\( _\gamma \) in the hematopoietic system, established that this molecule is expressed in neoplastic conditions, and demonstrated in the k-562 leukemic cell line that PTP\( _\gamma \) expression is associated with a selective inhibition of erythroid differentiation.
Understanding the repopulating characteristics of human hematopoietic stem/progenitor cell fractions is crucial for predicting their performance after transplant into patients receiving high-dose radio-chemotherapy. Based on the previous demonstration of the potent mitogenic effect of FL3 ligand (FL), stem cell factor (SCF) and thrombopoietin (TPO) on cord blood stem cells, we chose to focus on these cytokines without or with the addition of IL6 and IL3, to investigate their effect on the proliferation, the self renewal potentials and the manipulation. CD34+ cells were cultured in stroma-free suspension cultures in IMDM containing either 10% FCS or pooled human sera in the presence of various combinations of FL, SCF, TPO, IL6, IL6R and IL3. Cultures were either semi-depopulated every week by removal of half culture volume, which was replaced by the same amount of fresh medium and growth factors or doubled every week by adding the same volume of fresh medium and growth factors. Cells of each set of wells were harvested weekly, counted and aliquots were kept for assessment of immunophenotype, CFC and LTC-IC content. Of the various growth factor (GF) combinations many proved able to sustain a certain degree of cell production, which, after 6-7 weeks, ranged from 4.8×10^6 to 268×10^6 cells/well. When suspension cultures were extended for longer periods of time, only a few GF combinations, containing FL, SCF, TPO±IL6 proved capable of supporting prolonged and increasingly greater cell production, which persisted for up to 16 weeks and reached 125,000-fold the initial number. Accordingly, also the output of committed progenitors persisted and kept increasing up to 12-15 weeks (up to >7,000-fold). Limiting dilution analyses showed that not only LTC-IC persisted, but also underwent a certain degree of expansion (up to 5 weeks). To demonstrate that the expanded cells also retained their in vivo repopulating ability, sublethally irradiated NOD/SCID mice were injected with either decreasing concentrations of unmanipulated CD34+ BM and MPB cells or the corresponding progeny of initial 40 to 80×10^3 CD34+ cells which were expanded in vitro for up to 3 weeks. Cyt fluorimetric and DNA analysis of the BM cells of the engrafted animals showed that SRC were not only maintained up to 25 days of culture, but also expanded after 2 to 3 weeks. However, in contrast to CB experiments, FL + TPO were necessary, but not sufficient to allow such an expansion. In addition to these two growth factors, other growth factors, among which SCF, IL6, IL6/IL6R and also very low doses of IL3, seem to be important for SRC maintenance and expansion of BM and MPB more primitive stem cells.

PO109
EX VIVO EXPANSION OF TRANSPLANTABLE PRIMITIVE STEM CELLS OF ADULT HEMATOPOIETIC TISSUES
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A molecular based capillary electrophoresis method was used to study heavy chain immunoglobulin (IgH) gene rearrangement and to detect minimal residual disease (MRD) in multiple myeloma (MM). The method includes: 1) PCR amplification using two sets of sense primers from the IgH leader region and FRI respectively and a unique antisense fluorescent primer [H]; 2) identification of a monoclonal band by capillary electrophoresis using the GeneScan 3.1.2 ABI PRISM 310 program; 3) sequencing of VDJ rearrangement to identify the hypervariable domains; 4) synthesis of a CDRIII sense primer and a CDRIII fluorescent antisense primer for each patient. Thirty-four patients were recruited for this study, DNA was isolated from bone marrow aspirates collected at diagnosis. In two patients DNA was obtained after initiation of treatment. Monoclonal rearrangement was successfully detected in 23/34 patients (67%), including the two patients enrolled after chemotherapy. In 22 samples the clone specific part of the nucleotide sequence of CDRIII was identified by the forward primer, in one case the sequence was obtained.
by cloning. The sensitivity of this method has been tested in 10 patients, using the two specific primers constructed. For each patient three different serial dilution (10^−3 - 10^−5) were prepared by diluting DNA at diagnosis with DNA from a pool of normal donors. PCR conditions were optimized to avoid any background from control DNA. The sensitivity achieved was 10^−4 - 10^−5 in accord to that reported in previous studies, employing ASO-primers in a nested PCR technique. Patients underwent 2-3 VAD courses; after peripheral blood stem cell (PBSC) mobilization with cyclophosphamide (4-7 g/m²) patients were autografted by melphalan (200 mg/m²) conditioning and infusion of PBSC (>4 x 10^9/kg). After the treatment only three patients achieved complete remission (CR) with disappearance of serum monoclonal protein and in only one a short-lived molecular remission was assessed. The use of fluorescent primers allows us: to discriminate immediately between mono, oligo and polyclonal samples; to identify the size of monoclonal rearrangement with a variability of 1-2 nucleotides; to obtain a high sensitivity using lower amount of DNA (500 ng vs 1 mg) in comparison to that previously reported; to avoid cross-contamination risk associated with the nested-PCR strategy and simplify procedures; to detect monoclonal rearrangement even in those patients in whom treatment partially reduced the neoplastic clone. This method could prove effective also in monitoring minimal residual disease in lymphoma and leukemia.

PO111
HUMAN TELOMERASE REVERSE TRANSCRIPTION SPLICING VARIANTS IN UMBILICAL CORD BLOOD

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The human telomerase reverse transcriptase (hTERT) adds telomeric repeats to the ends of human chromosomes playing a key role in the long-term proliferative capacity of stem cells. The extent of the telomere shortening and the role of the telomerase need to be investigated to better understand the stem cell biology and its implication for stem cell transplantation. hTERT expression is closely linked to telomerase activity and may be regulated by different factors in different cellular contexts such as post-transcriptional control, alternative splicing and post-translational phosphorylation of hTERT protein. Six alternative splicing variants have been described corresponding either to insertion or deletion sites. To investigate possible mechanisms of regulation we examined the expression of hTERT in stem cells from umbilical cord blood and mobilized-peripheral blood stem cells. Total RNA was extracted from cord blood samples and leukapheresis products. Messenger RNA was reverse transcribed into cDNA and then amplified with specific primers for hTERT gene by polymerase chain reaction. The presence of amplifiable RNA was confirmed in all samples by RT-PCR of porphobilinogen deaminase gene RNA. The amplified products were separated on agarose gel. Telomerase gene expression analysis in umbilical cord cells showed the presence both of full length hTERT and variant transcripts. Thirty percent of the examined cord blood samples expressed shorter message transcripts that may correspond to the a-deleted transcript missing conserved residues from the catalytic core of the protein. In peripheral blood stem cells mobilized from patients with hematological malignancies the full-length message was the only transcript detected. The clonogenic capacity of the CD34+ cells was also evaluated in order to assess the relationship with hTERT variant expression. Preliminary data show no difference in the percentage of granulocyte-monoocyte colony-forming units obtained from cord blood expressing either full-length or variant transcripts. A potential implication of hTERT transcript variants in the biological clock of stem cells can be drawn from our observations and it may be important in understanding telomerase regulation during development and differentiation.

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PO112
ANTIANGIOGENIC ACTIVITY OF THALIDOMIDE IN LEUKEMIC CELL LINES AND CELLS OF PATIENTS WITH MYELOFIBROSIS

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The antitumor activity of thalidomide was evaluated in vitro in the HEL erythroleukemic cell line and in peripheral blood mononuclear cells (PBMCs) of 7 patients with myelofibrosis with myeloid metaplasia (bone marrow cells were available only in 1/7 cases). Proliferation tests, performed with colorimetric method (WST-1), showed that thalidomide, at concentrations of 10, 20 and 30 µg/mL, had no cytotoxic effect on the cell line HEL. The modest inhibition observed for PBMCs of patients with myelofibrosis was probably due to the high DMSO concentration necessary to resuspend thalidomide and not to thalidomide (p>0.05). Hemopoietic factors (Tpo, FLT3-ligand, HGF) and thrombin produced different effects on mRNA expression of VEGF and TGF-β in HEL. Only thrombin induced high levels of the two angiogenic factors as shown also by the analysis of concentrations in cell supernatants (ELISA). Thalidomide was not able to inhibit VEGF production; on the contrary, in some cases, the synthesis of the angiogenic factor was found increased. On the other hand, thalidomide strongly reduced the biological effects of thrombin on TGF-β synthesis. It would be interesting to determine the mechanism by which thalidomide interfere with thrombin action. In the case of myelofibrosis PBMCs, and above all of CD34+ progenitors, experimental data showed, with an exception, significantly higher baseline expression of VEGF, TGF-β and pFGF in neoplastic cells in comparison to cells of normal donors. Again baseline serum levels were higher than in control samples. PBMCs, incubated with thalidomide (10 mg/mL) for 24 h, showed a significative reduction of TGF-β but not VEGF mRNA, in treated cells compared to controls. In conclusion, thalidomide did not seem to interfere directly with the synthesis of the major angiogenic factor, VEGF. On the contrary, our model suggests a role of thalidomide in the regulation of TGF-β 1 which is a known VEGF inducer.
HMGA2 rearrangement in a case of acute lymphocytic leukemia

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The HMGA-2 gene is a member of the high mobility group proteins which are non-histone nuclear proteins involved in the regulation of chromatin structure. They are thought to affect transcription by acting as architectural proteins. We have recently reported a case of Richter transformation of a CLL with a 12q13 translocation involving the HMGA2, the breakpoint identified by FISH was in the third intron (major breakpoint region). This is the first case of a hematologic neoplasia in which a rearrangement of the HMGA-2 was shown to exist. In order to obtain more information about the involvement of this gene in leukemia, we analyzed one case of acute lymphatic leukemia showing a translocation concerning the 12q13-15 chromosomal region by FISH using BAC clones encompassing the entire HMGA-2 gene. Methods. Chromosomal preparations were made from 24-hour cultivated bone marrow (BM) samples and used either for the cytogenetic analysis or for FISH: to identify whether HMGA-2 BAC probes for the 5' and 3' ends were hybridized on metaphase spreadsings. The BAC 698I6 covers the first three exons while 669G18 spans the third intron and exons 4 and 5 (courtesy by Prof. A. Fusco). HMGA-2 gene expression was analyzed by RT-PCR. Immunohistochemical analysis was performed from BM smears with antibody against the recombinant HMGA-2 protein. Results. In a patient affected by ALL with t(4;11), a 46,XY,t(4;11) (q21;q23) t(9;12)(p22;q13) karyotype was found when relapsed. FISH analysis with BAC 69816, that covers the 5' region of the HMGA-2 until the third exon, showed three signals, one signal on the normal chromosome 12, one on der(12) and one on the der(9). HMGA-2 gene expression was analyzed by RT-PCR using two couples of primers: the first couple included primers on the first and fourth exons, the second on the first and third exons. In the first case an abundant expression was observed, whereas with the second couple of primers no band was detected. This result indicates a breakpoint in the HMGA-2 gene at the level of the third exon. Using the antibodies against the recombinant HMGA-2 the BM smear was analyzed by IHS for HMGA-2 expression. No immunoreactivity was observed in the normal lymphocytes, whereas cytoplasmic and perinuclear immunostaining was present in the blasts. Discussion. Herein we report on molecular and FISH analysis of a t(4;11)ALL, that showed in progression a translocation t(9;12)(p22;q13). The FISH analysis demonstrates that the breakpoint on chromosome 12q is located in the HMGA-2 gene in the third exon. Immunohistochemical analysis performed on the BM smear demonstrated the expression of the HMGA-2 protein specifically in the blasts. The overexpression of the HMGA-2 gene is casually related to cell trasformation and is a consequence of HMGA-2 rearrangement. The finding of HMGA-2 rearrangement in LA not only widens the spectrum of disorders that may be associated with altered function of this gene, but also provides insights about prognosis. The aberrant expression of the HMGA-2 gene by IHS in the blasts but not in the normal cells gives further support to the hypothesis that the HMGA-2 rearrangement might have a role in the process of leukemogenesis.
Translocations involving chromosome band 8p11 are associated with a peculiar subtype of AM1 characterized by blast cells of monocytic phenotype and erythrophagocytosis. After the first cloning of the (t(8;16)(p11p13) which showed the fusion of the monocytic leukemia zinc finger protein (MOZ) to the CREB-binding protein (CBP) gene, fusion of MOZ with p300 histone acetyltransferases was shown in a case of acute monocytic leukemia with (t(8;22)(p11q13). Very recently, fusion of MOZ with the nuclear receptor cofactor TIF2 was demonstrated for the first time in two cases of AM1 with inv(8)(p11q13). MOZ,TIF2 and CBP are proteins involved in acetylation processes through histone acetyltransferases (HAT5) activity. We describe a third case of a 21-year-old girl affected by AML M5a with inv(8)(p11q13) in whom the fusion gene between MOZ and TIF2 was identified. Cytogenetic study revealed inv(8)(p11q13) in 10 of 20 analyzed metaphases with otherwise normal XX karyotype. The correponding gene fusion was characterized by PCR technique, showing a breakpoint occurring at nucleotide 3744 of MOZ (corresponding to amino acid 1117) and nucleotide 2794 of TIF2 (corresponding to amino acid 939). The fusion product retains the C4HC3 and C2HC zinc fingers, the HAT domain, the MYST (corresponding to amino acid 939) The fusion product retains the C4HC3 and C2HC zinc fingers, the HAT domain, the MYST domain and the CBP interaction domain of TIF2. Additionally, internal tandem duplication of TFL1 was detected by molecular techniques. Bone marrow examination showed massive infiltration of blast cells characterized by prominent nucleolus, fine cytoplasmic granulation and vacuolization. Moreover, erythrophagocytosis was present. Immunophenotype of the blast population was as follows: CD13 69%, CD33 92%, CD15 82%, CD56 83%, CD4 93%, CD36 81%, CD34 5%, CD14 25%, anti MPOX 15%. The clinical course was characterized by resistance to conventional chemotherapy and fatal progression of disease despite allogeneic bone marrow transplantation from the compatible sister. This case supports the concept of a common molecular mechanism related to AML, suggesting the potential need for more specific pharmacologic targeting of this heterogeneous disease. However, in the case of AML with inv(8), as a consequence of the mutation of the histone acetylases MOZ, p300 and CBP, transcriptional repression of the target genes through recruitment of the repressor apparatus including histone deacetylases may be the ultimate event leading to leukemogenesis. Clinical trials with histone deacetylase inhibitors are currently underway to test this hypothesis.
Analysis of T-Cell Receptor Beta Chain Repertoire by CDR3 Spectratyping Demonstrates the Clonality of the Disease as Well as the Presence of Additional T-Cell Clones in T-Cell Chronic Lymphoproliferative Disorders

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Human T-cell chronic lymphoproliferative disorders are heterogeneous clonal proliferations of T-cells in which the rearranged T-cell receptor (TCR) can be considered a membrane tumor-specific antigen. Here we describe the molecular aspects of the TCR-Vβ repertoire rearrangement in two T-cell chronic lymphocytic leukemia (T-CLL) and in two T-cell prolymphocytic leukemia (T-PLL) patients. The TCR-Vβ repertoire was analyzed on peripheral blood lymphocytes (PBL) by the CDR3 Spectratyping which is a RT-PCR based technique that, determining the heterogeneous size length of the complementarity determining region 3 (CDR3) in each of the 24 TCR-Vβ subfamilies, allows to evaluate the individual expression of the repertoire complexity as well as definition of TCR-Vβ clonality. All patients were examined at diagnosis and one of them at clinical remission too. A major CDR3 clonal rearrangement was found in the patients in different TCR-Vβ sub-families, in particular in the Vβ 15 and Vβ 20 in the CLL and in the Vβ 3 and Vβ 7 in the PLL cases. These molecular data were confirmed by flow cytometric analysis in two patients; in the T-CLL patient with clonality in Vβ 15 the MoAb was not available. In the fourth case (T-PLL) the leukemic tumor cells did not express mCD3, CD4 and CD8 and thus might have been derived from a more immature T-cell proliferation. In all cases the CDR3 Spectratyping showed further TCR-Vβ clonal expansions different from the major one. One patient with T-PLL who achieved a clinical and hematological remission after therapy with Campath 1-H, still showed the presence of minimal residual disease at molecular analysis. Interestingly in this case additional Vβ clonal expansions were present at clinical remission too and two of them involved the Vβ 4 and Vβ 15 subfamilies which had been found clonally expanded at diagnosis. The PCR products of these two subfamilies were directly sequenced and the nucleotide analysis showed the same sequences for both at diagnosis and at remission. These data demonstrate that CDR3 Spectratyping is able to disclose both the major TCR-Vβ rearrangement, specific to the neoplastic cells, as well as additional TCR-Vβ clonalities. The functional role of these minor TCR clones remains to be defined. Multiple clonal T-cell expansions have been identified in normal subjects as well as in different pathological conditions. Most of the studies conclude that they probably represent an antigen-driven proliferation. In our case the two recurrent TCR transcripts (i.e., with identical junctional region) identified at diagnosis and at clinical remission, may be expression of a persistent tumor-associated antigen stimulation. Further in vitro functional studies are necessary to show whether these T-cell clones specifically recognize autologous tumor cells.

Molecular Monitoring of Chronic Granulocytic Leukemia after Allogeneic Bone Marrow or Peripheral Blood Stem Cell Transplantation

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The BCR-ABL rearrangement is the hallmark of chronic granulocytic leukemia (CGL) and is used to identify minimal residual
Commitment experiments demonstrated that WEB 2086-treated MELC underwent a true induction of terminal division with activation of the erythrodifferentiation program including increased expression of α- and β-globin genes, and downregulation of c-Myc. This pattern closely recalled that of HMBA-induced MELC although, on a molar basis, induction efficacy of WEB 2086 vs. HMBA was approximately 5-fold higher. The process of MELC induced differentiation by WEB 2086 did not seem to involve histone H4 acetylation and could be efficiently reverted by the presence of either phorbol 12-myristate 13-acetate or c-PAF. Moreover, WEB 2086 proved to be a powerful inducer of erythroid differentiation also in the human leukemic cell lines K562 and HEL while MELC made resistant to HMBA were similarly unresponsive to induction with the PAF antagonist. The novel cytodifferentiation activity of WEB 2086 indicated that this compound, although structurally distant from any other known inducers, can be successfully employed in vitro for the commitment of erythroleukemia cells to terminal division and, prospectively, also in vivo as a potential anticancer agent due to its unique ability of triggering cell differentiation and contemporaneously antagonizing multiple positive effects of PAF on tumor growth, cell spreading, and angiogenetic process.

PO121
HOMING AND SURVIVAL OF THYMIDINE KINASE TRANSDUCED T LYMPHOCYTES INTO NOD/SCID MICE

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The use of Herpes simplex virus thymidine kinase (HSV-tk) gene, which confers a ganciclovir (gcv)-specific sensitivity to the transduced cells, could be a valid way to obtain a controlled graft-versus-leukemia (GvL)/graft versus host disease (GvHD). Human T lymphocytes were engineered with an LSN-tk retroviral vector encoding tk and neomycin resistance (NeoR) genes. In brief, 80×10^6 tk+ lymphocytes were injected intraperitoneally in NOD-SCID mice, with no acute toxicity. Engraftment was evaluated by human CD45+/CD3+ cytokurometric analysis and NeoR-based PCR performed on peripheral blood, bone marrow, liver, thymus and spleen at day +5. After 14 days gcv (10 mg/kg daily) cytokurometric analysis and PCR were repeated (day +19). Immunohistological studies with an anti-CD3 MoAb followed by APAAP staining were also performed on spleen and liver at the same time-points. Cyturometric studies showed the engraftment of human CD45+/CD3+ cells in all tissues at day +5. Immunohistological staining showed human CD3+ cells were present in the spleen and liver of the injected mice at day +5. In particular, lymphocytes home to the white pulp T cell area and to the red pulp; localization in the liver is prevalently in the periportal area. PCR confirmed cytokurometric and immunohistological results. After gcv treatment (day +19) cytokurometric analysis showed very few CD3+ cells and scattered human CD3+ cells were detected by immunohistology. PCR identified the transgene in 22% of all tissues samples (only thymic and splenic samples were positive). GvHD did not occur in any animal. These
Evidence for the aberrant gene expression of a new group of tumour antigens known as cancer-testis antigens (CTAs) is well-recognized in some solid tumors. Their selective normal tissue expression makes them ideal antigens for immune targeting of malignant disease. However, few data are reported about their expression in malignant hematologic diseases and their clinicoprognostic implications. In this study, we screened the mRNA expression of the cancer germ-line genes NY-ESO-1, MAGE-1, MAGE-3 and the tumor-overexpressed gene PRAME in peripheral blood mononuclear cells (PBMN) of 31 untreated patients (18 M / 13 F) affected by various hematologic malignancies. This series included 15 pts with B-chronic lymphocytic leukemia (CLL) 8 pts with acute myeloid leukemia (AML) (3 pts M2, 2 pts M1, 2 pts M4, 1 pts M7), 4 pts with myelodysplastic syndrome (MDS), 4 pts with chronic myeloid leukemia (CML) (in chronic phase and 1 in blast crisis), and they were compared to PBMN of 6 healthy control subjects. By reverse transcription- polymerase chain reaction (PCR) we found that transcripts for PRAME were detected in 70% (6+/8) of AML patients and, only in CML in blast crisis, but not, in chronic phase. None of the patients with AML or CML showed expression of NY-ESO-1, MAGE-1, MAGE-3. Moreover, none of the studied antigens was detected in CLL, MDS as well as in normal PBMN. Although the precise clinical significance of our findings remains to be determined, this study provides a basis for further investigations to characterize these antigens in acute myeloid leukemia.

PO23
MOLECULAR CHARACTERIZATION OF β-GLOBIN DEFECTS IN THE PROVINCE OF CASERTA: A PRELIMINARY STUDY

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The epidemiological data coming from the results of the screenings conducted in schools have disclosed a higher incidence of the globin β gene in the province of Caserta than the regional average. The Department of Hematology and the Centralized Service of Molecular Biology undertook, for about one year, a study for the search of the molecular defects responsible for ß-thalassemia trait. It is a preliminary study, within a vast project, to establish the incidence of the thalassemias and hemoglobinopathies in this territory. In one year we analyzed 175 chromosomes selected with the tests of first level, of these 72 are confirmed ß-trait. The investigation molecular behaviour with technical ARMS (amplification refractory mutation system) and MARMS (multiplex amplification mutation refractory system) procedure concluded: C 39 in 23 cases (equal to 32%), IVSI-nt 1 in 18 cases (equal to 25%), IVSI-nt 110 (equal to 14%), IVSI nt 6 in 5 cases (equal to 7%), IVSI-nt 1 in 2 cases (equal to 3%), -101 in 2 cases (equal to 3%), -87 in 1 case (equal to 1%). Three cases of association β* 39 and α +27 and two cases of intermediate-thalassemia for mixed heterozygotes IVSI-nt 110 and -87; b∞ 39 and -87. During the study we identified some hemoglobin variants (Hb D, Hb S, Jose and Hb Toulon) and 10 cases of Hb Lepore of which two homozygotes. Finally we detected 15 cases of α-thalassemia and of these 14 characterized as α - 3.7.6 homozygotes and one as - α-MED. A case is still in study for presumed g-thalassemia. These preliminary data in comparison with that written in literature establish a discordant element, represented by the higher incidence of the mutation IVSI-nt 1 in the province of Caserta. It is possible to reflect upon a natural selection (malaria?) or the transfer in a selected population in a determined geographical zone. From the data so far available it is deduced that the search of 9 mutations allows an effective and thorough enough genetic screening for an opportune diagnosis. The genotype-phenotype comparison permitted a medical genetics more effective to a prenatal diagnosis.

PO24
MLL IS NOT REARRANGED IN A T(2;11)(P21;Q23) IN A CASE OF T-AML/MDS

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The t(2;11)(p21;q23) is a rare aberration described in about 20 cases of MDS and de novo acute leukemia (AML and ALL) or following an MDS in patients over 50 years. A male gender seems to be predominant. The majority of patients have MDS (14), 2 have sideroblastic anemia, 5 RA, 4 RAEB. Six of them transformed to AML. Four other patients presented with AML (M0 which evolved to M4, M1, M2, and atypical M3). The prognostic implications of this translocation cannot yet be evaluated because of uncertainty about the breakpoints and the small number and the clinical heterogeneity of the patients. Cytogenetic attention is always focused on chromosome 11 at band q23, due to the presence of the MLL gene (ALL1, Htrx, HRX) which is involved in several translocations with different partners. Usually translocations of MLL disrupt the gene upstream of the regions coding for the zinc finger sequence motifs of the protein product. The 3' sequences of the gene are translocated or lost and the 5' regions are juxtaposed to the coding sequences of the genes of its translocation partners. In the translocation (2;11) the role of MLL is still not clear because only 2 out of 3 cases studied at the molecular level showed rearrangement of the gene, suggesting the possibility of a new gene involved in the breakpoint. We describe a 66-year-old patient with a diagnosis of AML-M5 evolved from an
MDS (CM M0 L) after 2 years. At the moment of acute leukemia diagnosis he presented with WBC=53×10^9/mL, Hb=10.3 gr/dL, PLT=209×10^9/mL. The cytogenetic analysis at the time of evolution of the disease showed a karyotype: 46, XY[1], 46, XY, t(2;11)[p21;q23][14]. The patient achieved a partial remission after induction therapy with cytosine arabinoside for seven days and daunoxome for three days. The patient was still alive with disease at 1 year from diagnosis, at last follow-up. We performed FISH with a dual color break-apart probe (Vysis) for the 3′-5′ MLL region. No rearrangement of the gene was detected in a total of 5 abnormal metaphases, DAPI counterstained, since fusion signals (2F) were detected on the normal and on the derivative 11. Furthermore, an additional 300 nuclei were analyzed, showing the normal 2F signals. This case is important because it contributes to show that another gene, which is disrupted or deregulated in the t(2;11), maps in this region.

**PO125**

**PHARMACOGENETIC DETERMINANTS OF RESPONSE TO CYTOTOXIC PURINE ANALOGS IN PATIENTS WITH CHRONIC LYMPHOPROLIFERATIVE DISORDERS**

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Chronic lymphoproliferative disorders are currently treated with purine analogs, including fludarabine and 2-chlorodeoxyadenosine. Cellular uptake of chemotherapeutic agents is allowed by extracellular dephosphorylation by ecto-5′-nucleotidase (ecto-5′-NT) followed by activation to cytotoxic metabolites by deoxyctydine kinase (dCK), while drug inactivation occurs by dephosphorylation through endo-5′-nucleotidase (endo-5′-NT). Since the enzymes of purine metabolism are critical determinants of chemosensitivity of malignant cells, this study assessed the expression of dCK, endo-5′-NT and ecto-5′-NT in lymphoid cells from patients with lymphoproliferative disorders. Patients with low grade non-Hodgkin’s lymphomas (LG-NHLs, n=28), B-chronic lymphocytic leukemia (CLL, n=14) and patients (28.5%). In the blood donors, endo-5′-NT was detectable in all samples (100%), dCK in 19 samples (68%) and ecto-5′-NT in 15 samples (54%), while the three enzymes were simultaneously detectable in 14 blood donors (50%). The dCK/endo-5′-NT ratio in CLL vs. LG-NHLs was 1.44±0.40 vs. 0.53±0.22 (p<0.05), while dCK+ecto-5′-NT/endo-5′-NT in CLL vs. LG-NHLs was 2.79±0.59 vs. 1.44±0.27 (p<0.05), dCK/endo-5′-NT ratio in blood donors vs. LG-NHLs was 0.98±0.68 vs. 0.63±0.24 (p<0.05), while the ratio dCK/endo-5′-NT in blood donors vs. CLL and the ratio dCK + ecto-5′-NT/endo-5′-NT blood donors (1.85±0.64) vs. both CLL and NHL-LG were not statistically significant. The higher dCK/endo-5′-NT and dCK + ecto-5′-NT/endo-5′-NT ratios in CLL suggest a higher likelihood of response to purine analogs as compared to LG-NHLs, possibly allowing the prediction of individual sensitivity or resistance to treatment. These results provide evidence of the feasibility of routine pharmacogenetic analysis in chronic lymphoproliferative disorders for prognostic purposes, in order to select patients who are likely to respond to nucleoside analogs.

**PO126**

**CYTOGENETIC ANALYSIS AND FLUORESCENCE IN SITU HYBRIDIZATION STUDY OF A T(1;11)(P32;Q23) IN A PATIENT WITH ADULT ACUTE LYMPHOBlastic Leukemia**


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Recurring translocations involving 11q23 are of great interest in acute leukemias and the MLL (myeloid lymphoid leukemia, ALL1, HRX, HTRX) gene has been found rearranged with more then 40 different partner genes, 20 of which have been cloned. The result of the translocation is a chimeric gene on the rearranged chromosome 11 consisting of the 5′ region of MLL and the 3′ region of the partner gene. Here we describe an interesting case of a 50-year old female patient presenting with acute lymphoblastic leukemia. Cytogenetic analysis of metaphase cells obtained from 48-hour cultures showed a female karyotype with a t(1;11)(p32;q23) [15]. FISH analysis was performed as suggested by the manufacturer using an MLL dual color break-apart probe (Vysis) to detect the rearrangement. The interphase analysis of 700 nuclei showed a deletion of the 3′ part of the MLL gene in a total of 608 nuclei (87%), appearing as a fusion signal (F) on the normal chromosome 11 and a green signal (G) on the der(11). Deletions of the 3′ part of MLL have been reported in about 20% of MLL translocation, the prognosis being controversial. t(1;11)(p32;q23) has been described in about fifteen cases in the literature in biphenotypic leukemia and in ALL as sole abnormality, in AML as sole abnormality or part of a complex karyotype, and a case of MDS RA has been described with this translocation as sole abnormality. In only two of the cases FISH or a molecular study was done, showing an MLL rearrangement at 11q23. The partner gene of MLL involved at 1p32 in the t(1;11) has been cloned: AF1p (ALL fused gene from chromosome 1p). The predicted wild type AF1p product is a 98 Kda acidic protein which does not exibit similarity to the AF4, AF9 and ENL gene products. AF1p seems to be implicated in endocytosis and the EGF receptor tyrosine kinase seems to function as substrate. In conclusion we found a case of t(1;11) involving MLL as deletion of the 3′ part. This is a new modality of involvement described in the literature for this translocation.
PO127
INTERPHASE FLUORESCENT IN SITU HYBRIDIZATION IS A RELIABLE METHOD TO DETECT BCR/ABL POSITIVE CELLS IN A CHRONIC MYELOID LEUKEMIA PATIENT PH NEGATIVE BY CYTOGENETICS AND BCR/ABL NEGATIVE BY RT-POLYMERASE CHAIN REACTION

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New FISH probes (Vysis) are now available, and they detect BCR-ABL fusion in interphase nuclei with a false-positive signal rate close to zero. We used cytogenetics, interphase fluorescent in situ hybridization (FISH), and reverse transcription-polymerase chain reaction (RT-PCR) methods to study minimal residual disease in a 48-year-old patient with CML in sustained complete cytogenetic remission 8 years after treatment with autologous bone marrow transplantation (ABMT) and in treatment with IFN-α. At the moment of the study the karyotype was 46, XX[30]. RT-PCR was negative for the BCR/ABL fusion transcript. The FISH study design was to analyze a bone marrow sample (BM) and two subsequent peripheral blood (PB) samples with or without previous incubation with GM-CSF. A total of 900 cells in interphase were scored for each sample by 3 different observers. Normal cells display 2 red signals and 2 green signals (2R2G), and abnormal cells 1 red, 1 green, and 1 yellow fusion signals (1R1G1F) or 2 red, 2 green, and 1 yellow fusion signals (2R2G1F). The positivity pattern for each specimen was recorded and final results were expressed as percentages of nuclei with fusion signals. The cutoff limit for BCR-ABL positivity was set at the mean of normal result (1%). Positive control was also performed with bone marrow transplantation (PBSCT) and two peripheral blood (PB) samples from patients with CML; it was suggested that the BCR-ABL translocation was present in the most primitive hematopoietic progenitor cells, which might have been quiescent and transcriptionally silent, possibly due to the molecular effect of IFN-α.

PO128
HIGH DOSE CHEMOTHERAPY WITH IDARUBICIN AND MELPHALAN AND PERIPHERAL BLOOD STEM CELL (PBSCT) TRANSPLANTATION IN PATIENTS WITH POOR RISK OR RELAPSED HIGH GRADE NON HODGKIN LYMPHOMA

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Idarubicin (IDA) is an active drug in several myeloid and lymphoid malignancies. In vitro and in vivo studies suggest that continuous infusion (c.i.) IDA has a better therapeutic index compared with bolus administration. However, few clinical data are available about c.i. IDA in the high dose chemotherapy (HDCT) setting and the timing of peripheral blood stem cell (PBSC) reinfusion is still controversial, due to the long serum half-life of its active metabolite Idarubicinol (OLO). Following our previously reported experience about IDA and OLO pharmacokinetics after c.i. IDA in humans, we now present clinical data about a new conditioning regimen for high grade NHL, that includes high dose c.i. IDA and melphalan (L-PAM). Twenty-eight NHL patients (M/F: 15/13, median age 46, range 19-61, 23 R cell and 5 T cell) were enrolled. Eleven patients (pts) were treated because of age-adjusted >2 IPI at diagnosis, 12 because of relapse after conventional chemotherapy, 16 because of high risk. Seventeen pts were stage III/IV, 11 presented with bulky disease and 10 with extranodal involvement. After CHOP-like induction, pts received 4 g/m² CTX and filgrastim to collect at least 2×10⁶ CD34+ cells/kg. According to response, pts received 2 other ESHAP cycles, before HDCT. IDA (15 mg/m²) was administered as c.i. from day -6 to day -4, L-PAM (180 mg/m²) on day -2 with PBSC reinfusion on day 0. From day +5, pts received filgrastim 5 mcg/kg and standard antimycotic, antibiotic and antiviral prophylaxis until hematological recovery. Grade 3 mucositis was observed in 39%, fever of undetermined origin in 64% and infection in 29% of cycles administered. One pt died because of pneumonia during aplasia; another pt died because of toxoplasma encephalitis on day +90, in complete hematological recovery. Median days to reach >500 PMN and >20000 PLT were 12 (range 1-33) and 11 (range 1-45) respectively. Pts received a median of 4 (range 2-14) and 2 (range 1-10) RBC and PLT units, respectively. Overall, 19 pts achieved a CR (67.5%) and 1 a PR (3.5%) with an ORR of 71%. After a median follow-up of 21.8 months (range 7-133), 2-year OS is 56%, with 53% of pts still in CR. Our results confirm that high dose c.i. IDA + L-PAM is effective and feasible for high risk or relapsed NHL pts. PBSC can be safely reinfused on day 6 without any engraftment delay, regardless of the high OLO levels expected. Our clinical results compare favorably with other widely used conditioning regimens for NHL pts.
SPINAL AND PERIDURAL ANESTHESIA IN BONE MARROW HARVESTS


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General anesthesia is generally used to harvest bone marrow, however this type of anesthesia may have some risk and is not comfortable for patients. On the other hand techniques of regional anesthesia have the disadvantage of hypotension, due to a combination of sympathetic block and to the volume of harvested bone marrow blood. We present here our experience in the use of regional anesthesia during procedures of bone marrow harvests in patients affected by hematological malignancies and in normal donors. Forty-two subjects underwent bone marrow harvests using regional anesthesia, 32/42 received spinal anesthesia while 32/42 received peridural anesthesia, mean age was 34 years, mean body weight 69 kg, 22 subjects were female and 20 were male, in 34 cases harvest was intended for autologous transplant and in 8 case for allogeneic transplant from normal donors. Mean time of anesthesia was 77.2 min. and mean volume of harvest was 1183 mL, mean cell concentration was 23.0×10^6/mL. No differences were apparent between the groups of harvests done using spinal or peridural anesthesia as far as TNC/mL and TNC/kg body weight were considered.

<table>
<thead>
<tr>
<th>Volume (mL)</th>
<th>CNT ×10^6/mL</th>
<th>CNT ×10^6/kg</th>
<th>Hypotension</th>
<th>Vomiting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinal anesthesia</td>
<td>1187</td>
<td>24.</td>
<td>3.9</td>
<td>30%</td>
</tr>
<tr>
<td>Peridural anesthesia</td>
<td>1168</td>
<td>22.</td>
<td>3.7</td>
<td>14%</td>
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Hypotension in the group of patients who underwent peridural anesthesia was less frequent in respect to the group who underwent spinal anesthesia (14% versus 30%), in all cases, however, hypotension was mild and responded to treatment. The group who underwent peridural anesthesia showed also a lower frequency of vomiting and a lower need for analgesia, mean hospital stay was 77.2 min. and mean volume of harvest was 1183 mL, mean cell concentration was 23.0×10^6/mL. No differences were apparent between the groups of harvests done using spinal or peridural anesthesia as far as TNC/mL and TNC/kg body weight were considered.

HIGH DOSE THERAPY AND AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN HIV-ASSOCIATED LYMPHOMA

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Peripheral blood stem cell transplantation (PBSCT) is a therapeutic approach of well known efficacy and widely used in lymphoma of the general population. For refractory or relapsed HIV-associated lymphoma (HIV-Ly) there are very few possibilities of cure with standard chemotherapy, but high dose therapy (HDT) and PBSCT has been considered prohibitive for long time in this setting, due to the immunodeficiency of patients and the high risk of infection. With the recent introduction of highly active antiretroviral therapy (HAART), immunological reconstitution became possible in these patients, making suitable evaluation of an aggressive treatment strategy with PBSCT also in HIV+ subjects. Three consecutive patients with poor prognosis HIV-Ly at our Institute started a program of peripheral blood stem cell mobilization and harvest, after evaluation of chemosensitivity with a brief course of conventional second-line chemotherapy. All the patients had Hodgkin's lymphoma. The first patient was in second relapse, the second patient in first early relapse (<1 year from remission) and the third patient had disease progression after partial remission with first-line treatment. All the patients were under HAART, with non-detectable HIV viral load, which was maintained during the procedures of PBSCT harvest and transplantation. From the first patient 8.3 ×10^6/kg CD34+ cells were collected after mobilization with cyclophosphamide 4 g/m^2 + G-CSF. The second patient failed to mobilize an adequate number of CD34+ cells after cyclophosphamide 4 g/m^2 + G-CSF and during hematologic recovery after salvage chemotherapy. From the third patient 7.0×10^6/kg CD34+ cells were collected at recovery after salvage chemotherapy. No adverse events were registered during the procedures of mobilization and harvest. Thus the first and the third patients underwent HDT according to BEAM (BCNU 300 mg/m^2; VP16 800 mg/m^2; Ara-C 800 mg/m^2; melphalan 140 mg/m^2) and PBSCT. In both cases a prompt hematologic recovery was observed, with neutrophil count >500/mm^3 at +10. The first patient experienced, as treatment-related toxicity, only a mild oral mucositis and he is in complete remission at 2 months from PBSCT. The third patient experienced severe (grade 4) mucositis, which was responsible for interruption of HAART treatment, fever and positivity for cytomegalovirus (CMV) DNA in the serum, with possible reactivation of a previous CMV chorioretinitis; now he is doing well at day +20 after PBSCT. An aggressive treatment approach with PBSCT is feasible in HIV-Ly, with the possibility of collection of an adequate number of CD34+ cells, rapid recovery after PBSCT and a non-prohibitive regimen-related toxicity. The clinical efficacy and the impact on HIV infection status need to be evaluated in a wider series of patients. Severe mucositis with possible incapacity to take HAART is a problem that deserves attention.
while those with Hodgkin’s disease (HD, n=21) received the IGEV regimen/G-CSF (ifosfamide 2000 mg/m²/d as 2-hour iv infusion plus mesna 2600 mg/m²/d iv on days 1 to 4; gemcitabine 800 mg/m²/d iv short infusion on days 1 and 4; vinorelbine 20 mg/m²/d iv bolus on day 1; prednisolone 100 mg/d iv on days 1 to 4). Most patients were heavily pretreated with radiotherapy and different chemotherapy regimens. The target yield was > 3 ×10^6 CD34+ cells/kg of body weight, in order to support the subsequent myeloablative chemotherapy. Results: The optimal time of peripheral blood progenitor cell (PBPC) harvest was on days 11 and 12 with no differences in the PB CD34+ cells mobilization kinetics between the two regimens. The median number of collected CD34+ cells per kilogram of body weight was 10.9 ×10^6 (range 1.7-6×10^6). The median total CD34+ cell/mL, CFU-GM and WBC for all individual sets of collection was respectively 87, 110 ×10^6/kg, and 16,400. The PBPC target yield was achieved in 28 of the 31 patients. Hematological side-effects were acceptable and no treatment-related hospitalization or toxic death was observed. Twenty patients have subsequently received high-dose therapy with rapid engraftment. Neutrophil recovery was achieved after a median of seven days (range 6-11) and the median time to platelet recovery was 14.5 (range 0-106). No correlation between the amount of CFU-GM, CD34+ cells reinfused and days to engraftment could be observed. Interpretation and Conclusions. These results demonstrate that ifosfamide and vinorelbine-based chemotherapy regimen with G-CSF can be successfully and safely used to mobilize PBPC.

PO132
TANDEM AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN HIGH-RISK RELAPSED OR REFRACTORY LYMPHOMA: PRELIMINARY RESULTS OF A PILOT STUDY

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The impact of modern supportive care that includes hematopoietic growth factor support, PBPCs (vs bone marrow) as the primary stem cell source, and newer antibiotics and antifungal agents have markedly decreased transplant-related toxicity. However, this has not translated into major improvements in overall survival of lymphoma patients with unfavorable risk factors at transplantation. In order to improve further the outcome of such patients we conducted a pilot study of tandem high-dose chemotherapy (HDC) consisting of Carmustine (600 mg/m², day -7), etoposide (200 mg/m², day -6, 5, 4, 3), cytarabine (400 mg/m², day -6, 5, 4, 3) e melphalan (140 mg/m², day -2) (BEAM) as first HDC, and etoposide (250 mg/m², day -7, 6, 5, 4), thiopeta (166 mg/m², day -6, 5, 4) and carboplatin (266 mg/m², day -6, 5, 4) (ETC) as second HDC. So far, three pts with Hodgkin’s disease (1 first CR, 1 first PR, 1 second PR) and five pts with high-grade non-Hodgkin’s lymphoma (1 first CR, 2 first PR, 2 second PR) have been enrolled. The median follow-up is 27 months (17–44) from the first transplant and 51 months (38-81) from diagnosis. There are no differences among engraftment kinetics and toxicity profile in comparison of the 1st with the 2nd HDC. No treatment related death was observed. Preliminary results on treatment effectiveness were satisfactory: 4 pts (50.0%) were in complete remission after the first and 7 (87.5%) after the second HDC. As of April 30 2001, all the pts are alive and 6 of 8 pts are in CR. This seems an improvement as compared to the 51% and 61% of progression-free survival and overall survival observed in our historical series including 57 pts treated with single HDC. In summary, we can conclude that tandem HDC with PSCT is feasible with tolerable toxicity and high rate of durable responses. This study was funded in part by AIL and Regione Calabria.

PO133
USEFULNESS OF BONE MARROW PURGING USING ASTA-Z BEFORE AUTOLOGOUS TRANSPLANTATION IN 1ST COMPLETE REMISSION ACUTE MYELOID LEUKEMIA PATIENTS

Divisione Clinizzata di Ematologia con Trapianto Ospedale Ferrarotto, Catania

Autologous Bone Marrow transplantation is the treatment of choice in AML patients in 1st CR aged less than 60 years who do not have a HLA identical sibling; however, it is followed by a high risk of relapse. Since relapses originate from leukemic cells contaminating the bone marrow harvests, the use of pharmacological agents, such as ASTA-Z, to purge marrow has a strong rationale. However the advantage of using purged over unpurged marrow, in autologous transplantation of AML patients, has been shown only in non-randomized studies. In our Institution 26 patients affected by AML in CR underwent ABMT using ASTA-Z purged or unpurged bone marrow, depending on availability of ASTA-Z. Seventeen pts (65%) received unpurged marrow while 9 pts (35%) received ASTA-Z purged marrow. These two groups of patients were comparable in mean age (41y. vs 39 y.), in the median time between diagnosis and transplantation (220 days vs 221 days), in the median number of harvested TNC (3.7 ×10^10/kg vs 3.2×10^10/kg) and in median follow-up (25 versus 31 months). All patients were treated using BuCy protocol and received the same supportive care. Engraftment in these two groups has been comparable and median time to reach a N>500 count was 25 days in purged marrow and 24 days in the group of unpurged marrow. Time to reach PLT> 20,000 was 120 days in purged marrow and 77 days in unpurged marrow. Requirement of platelet transfusions was not different in the two groups. No case of TRM has been registered. In the group of purged marrow 7/9 patients are alive in CR and DFS is 71% at 3 years while in unpurged marrow group 8/17 patients are alive and in CR and DFS is 39% at 3 years. Survival curves of these two groups are different at a marginally significant level (logrank test p=0.07). In this preliminary experience, in the treatment of AML in CR the use of ASTA-Z purged marrow in respect to unpurged marrow leads to an higher DFS with a comparable toxicity.

DFS
\[ N>500 \quad PLT>20,000 \quad CNT>38^\circ C \quad TRM \]
\[ \begin{array}{c|c|c|c|c}
\text{Purged} & 71\% & 25\% & 120\% & 3.2 & 6.8 days \quad 0 \\
\text{Unpurged} & 39\% & 24\% & 77\% & 3.7 & 2.8 days \quad 0 \\
\end{array} \]

Ferrara, Catania
Divisione Clinizzata di Ematologia con Trapianto Ospedale Ferrarotto, Catania

The impact of modern supportive care that includes hematopoietic growth factor support, PBPCs (vs bone marrow) as the primary stem cell source, and newer antibiotics and antifungal agents have markedly decreased transplant-related toxicity. However, this has not translated into major improvements in overall survival of lymphoma patients with unfavorable risk factors at transplantation. In order to improve further the outcome of such patients we conducted a pilot study of tandem high-dose chemotherapy (HDC) consisting of carmustine (600 mg/m², day -7), etoposide (200 mg/m², day -6, 5, 4, 3), cytarabine (400 mg/m², day -6, 5, 4, 3) e melphalan (140 mg/m², day -2) (BEAM) as first HDC, and etoposide (250 mg/m², day -7, 6, 5, 4), thiopeta (166 mg/m², day -6, 5, 4) and carboplatin (266 mg/m², day -6, 5, 4) (ETC) as second HDC. So far, three pts with Hodgkin’s disease (1 first CR, 1 first PR, 1 second PR) and five pts with high-grade non-Hodgkin’s lymphoma (1 first CR, 2 first PR, 2 second PR) have been enrolled. The median follow-up is 27 months (17-44) from the first transplant and 51 months (38-81) from diagnosis. There are no differences among engraftment kinetics and toxicity profile in comparison of the 1st with the 2nd HDC. No treatment related death was observed. Preliminary results on treatment effectiveness were satisfactory: 4 pts (50.0%) were in complete remission after the first and 7 (87.5%) after the second HDC. As of April 30 2001, all the pts are alive and 6 of 8 pts are in CR. This seems an improvement as compared to the 51% and 61% of progression-free survival and overall survival observed in our historical series including 57 pts treated with single HDC. In summary, we can conclude that tandem HDC with PSCT is feasible with tolerable toxicity and high rate of durable responses. This study was funded in part by AIL and Regione Calabria.
In this randomized study we compared two different peripheral blood stem cells (PBSC) mobilization schedules in 37 patients affected with lymphoma. Patients in the first arm were mobilized with G-CSF alone at the dosage of 10 µg/kg/die s.c., while patients in second arm were mobilized with cyclophosphamide (CTX) at the dosage of 4g/m² day 1 followed by G-CSF at the dosage of 10 µg/kg/die s.c. from day +5 to the end of harvesting. In order to avoid any bias due to uneven distribution of more heavily pre-treated patients, randomization was stratified at the dosage of 10 µg/kg/die s.c. from day +5 to the end of harvesting. To assess the efficacy of mobilization procedures we compared mean peak of CD34+ cells/mm³ obtained after CTX + G-CSF in not heavily treated lymphoma patients with two or less lines of chemotherapy treatment vs CTX + G-CSF (49.10 vs 55.77 CD34+ cells/mm³, t-test = 0.74).

No patient had bone marrow involvement at time of mobilization. To assess the efficacy of mobilization procedures we compared mean peak of CD34+ cells/mm³ obtained after mobilization. In this analysis we found that in patients previously treated with more than two lines of chemotherapy mean peak of CD34+ cells/mm³ obtained after CTX + G-CSF was significantly higher than after G-CSF alone (42.48 vs 10.26 CD34+ cells/mm³, t-test = 0.045). On the contrary, in patients previously treated with two or less lines of treatment mean peak of CD34+ cells/mm³ obtained after G-CSF alone was not significantly different compared to CTX + G-CSF (49.10 vs 55.77 CD34+ cells/mm³, t-test = 0.74).

In conclusion G-CSF alone may be used to obtain a satisfactory mobilization of PBSC in not heavily treated lymphoma patients. On the contrary, in the heavily treated ones, mobilization of PBSC using G-CSF alone has very little possibility of being successful.
bone marrow at HUMARA assay. Further controls documented the normalization of both clonality and cytogenetic study at 43, 48 and 67 months after SCT. The occurrence of MDS and sAML depends on a variety of risk factors including the number and type of prior chemo-radiotherapy regimens, total body irradiation in the conditioning regimen and cytogenetic and morphological alterations prior to SCT. This may account for the discrepancies in reporting MDS/sAML. In this study the absence of morphological or cytogenetic abnormalities prior to transplantation, the use of chemotherapy-based conditioning regimen and the disease status at SCT resulted in the absence of overt MDS/sAML. We also maintain that the use of stringent morphological criteria combined with cytogenetic, clonality and FISH results especially during the first period after SCT should be adopted in order to exclude the erroneous diagnosis of MDS.

PO126
AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

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High dose therapy (HDT) with autologous bone marrow transplantation (ABMT) is widely used in the treatment of acute myeloid leukemia (AML) as post-remission therapy for younger patients. In this study we report the results of HDT/ABMT in patients with AML enrolled in our center. Fifty-one patients (median age 35 years; female: 24, male: 27) underwent ABMT; they included 61% in first complete remission (CR1), 26% in second complete remission (CR2) and 13% in relapse. Preparative regimens consisted of busulfan (16mg/kg) and cyclophosphamide (CY) (120 mg/kg) or busulfan (16 mg/kg), CY (120 mg/kg) and etoposide (60 mg/kg). At the bone marrow harvest all patients were in complete remission and purging was performed in 39 patients with mafosfamide at a standard dose of 50 µg/mL (29) or individually adjusted (10). The median dose of mononucleated cells infused was 1.31×10^8/kg (range 1.8-3.66×10^8/kg) for CD34+ cells. Complete remission was reached in 74% of patients and the leukemia relapse was 30%. The median time to recovery of polymorphonucleated cells to 500/mm³ and of platelets to 50,000/mm³ was 22 days (range 12-73) and 39 days (range 15-232) respectively. The median overall survival (OS) was 39 days (range 15-232) respectively. The median overall survival (OS) was 20 months (range 6-174 months), 3-year estimates of overall survival, event-free survival, and disease-free survival were 47% (95% CI, 36% to 59%), 44% (95% CI, 34% to 54%) and 64% (95% CI, 50% to 74%), respectively. Multivariate analysis showed chemosensitivity to HDS to represent the strongest predictor of both CR and survival. This retrospective study shows that salvage treatment with HDS has a relatively low toxicity and is associated with remarkable response rates, allowing further effective therapy with high-dose autograft programs.

PO137
INTENSIFICATION OF SALVAGE TREATMENT WITH HIGH-DOSE SEQUENTIAL CHEMOTHERAPY IMPROVES THE OUTCOME OF PATIENTS WITH REFRACTORY OR RELAPSED AGGRESSIVE NON-HODGKIN’S LYMPHOMA

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Peripheral blood stem cell (PBSC) autologous transplantation for hematologic malignancies allows faster hematological recovery and shorter hospitalization. Using this procedure we collect and reinfuse a superior number of progenitors (CD34+) than by bone marrow harvest; however, the quality of the harvest is still matter of debate. Aim of this study was to evaluate whether stimulation with GM-CSF at a dose of 150 mg s.c. given for 3 days before bone marrow harvest (GM-BM) could improve quantity
and quality of CD34+ cells in comparison with non pretreated bone marrow harvest (BM), and whether this could affect hematological and immunological recovery in the post-transplantation period. We compared three groups of lymphoma patients who underwent autologous transplantation (BM vs GM-BM vs PBSC), evaluating reconstitution pattern, disease free survival (DFS) and overall survival (OS). From 1995 we autotransplanted 30 patients (18 male and 12 female); 19 non Hodgkin’s lymphomas (NHL) and 11 Hodgkin’s disease (HD). Eleven patients received autologous BM (median age: 34 y), 11 GM-BM (median age: 38 y) and 8 PBSC (median age: 34 y). At transplant, 17 patients were in complete remission and 13 in partial remission or with active disease. 11 patients had received one or two previous treatment lines, and 19 more than two. Median number of CD34+ cells harvested in the 3 groups, expressed as ×10^6/kg, were 0.6 for BM, 0.9 for GM-BM and 7.2 for PBSC. Hematological lymphocytes >1000/mmc and platelets > 20,000/mm3, showed hematological lymphocytes >1000/mmc and platelets > 20,000/mm3, showed overlapping values between BM and GM-BM and a faster recovery in the PBSC group, with statistical significance for platelet recovery. Immunological recovery evaluated 12 months after transplant showed different kinetics of CD4 and CD8 reconstitution between BM and GM-BM, with a faster and durable tendency to normalization of the CD4/CD8 ratio in the GM-BM group as compared to BM and PBSC. Outcome evaluation in terms of DFS and OS has shown an advantage for the GM-BM group (DFS 100% at two years) in comparison with BM (DFS 70%) and PBSC (DFS 40%) with statistical significance (p = 0.03) between GM-BM and PBSC. In conclusion, our preliminary experience suggests that co-stimulation by GM-CSF before bone marrow harvest does not significantly modify the number of CD34+ cells harvested, nor does improve hematological recovery in lymphoma patients; however, it seems favorable in terms of immunological recovery and survival. Further studies are needed to evaluate the role of pretreatment with GM-CSF on the bone marrow micro-environment and on the control of minimal residual disease after autologous transplantation.

PO139
RETICULATED PLATELETS AND GLYCOCALICIN DOSAGE IN AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Platelet recovery is usually the last step in hematopoietic engraftment after APBSCT. The amount of reticulated platelets shows bone marrow platelet productivity while glyocalcicin is linked to platelet turnover or destruction and to increased thrombocytopenia. These new tests allow a further look into thrombopoietic recovery. In our laboratory we have studied 10 patients undergoing APBSCT, measuring reticulated platelet number using a cytofluorimetric method and glyocalcicin using an ELISA test. Our results are shown in the table below. Our study results, even if with only 10 patients, underline the significance of reticulated platelets, along with glyocalcicin measurement as a predictive index of platelet recovery after APBSCT and as a useful guide in transfusion support of these patients. Actually we have noticed that reticulated platelets increase 2 days before platelet recovery. Further studies are underway to support these data and to identify groups of patients showing a different pattern of platelet recovery.

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PO140
AUTOLOGOUS TRANSPLANTATION FROM BONE MARROW vs PERIPHERAL STEM CELLS FOR ACUTE MYELOID LEUKEMIA IN FIRST COMPLETE REMISSION

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Autologous transplantation (AuBMT) from peripheral blood stem cells (PBSC) is characterized by a rapid hematological recovery. Because of shorter duration of neutropenia, reduced incidence of infectious complications and of supportive care, this procedure is going to replace autotransplantation from bone marrow (BM). It is still debated whether the procedures are equivalent in terms of disease-free survival (DFS) and overall survival (OS). The aim of our study was to address this question in a cohort of patients with acute myeloid leukemia (AML) autotransplanted in first complete remission (CR). From 1990, 30 AML patients underwent AuBMT, 18 from BM and 12 from PBSC. Median age was 42 years (range 22-58yrs) for the BM group and 39 yrs (range 16-61) for the PBSC group. According to FAB classification, the distribution in the two groups (BM/PBSC) was: M1: 1/5; M2: 9/3; M4: 4/3; M5: 2/1; M6: 1/0; hybrid: 1/0. Cyto-genetical risk was equivalent in the two groups. Median time from diagnosis to AuBMT was 6.1 months for BM and 5.8 months for PBSC. All patient were conditioned by BuCY-2 except 3 patients conditioned by BAVC because of age. Median cellularity reinfused was 0.6×10^6/kg for BM and 8×10^6/kg for PBSC. PBSC had a faster hematological recovery than BM (N>1,000/mm3 15 vs 27d; Plt>20,000/mm3 12 vs 44d) and a shorter hospitalization. We have recorded a higher number of relapses in the PBSC group (8/12= 66.6%) than in the BM group (5/18= 27.7%). DFS projected to 5 y was 70% in BM vs 30% in PBSC (p= 0.03). OS projected to 5 y was almost the same in the two groups. Thus, in this small series AuBMT from BM seemed to be superior than AuBMT from PBSC in terms of DFS. The same OS in the two groups is probably due to a minor number of deaths and a higher response to rescue therapy for the PBSC group.
In some hematologic diseases, it is difficult to find a suitable therapy for clinical remission induction and, at the same time, for CSC mobilization. In our Center we treated two young women of 35 and 22 years old affected by acute lymphoblastic leukemia L3 with CNS involvement (the first one) and by Burkitt’s Lymphoma (the second one). Since the first one was 5 months pregnant, we induced her to give birth by Cesarean section, and we treated both the patients with the polychemotherapeutic protocol 89-C-41 described by Magrath (4 alternative cycles: 2 CODOX-M and 2 IVAC). For each CODOX-M we administered: 1,600 mg/m² of CTX, 3 mg/m² of VCR, 40 mg/mg of adriamycin, 6,720 mg/m² of MTX and CNS prophylaxis with ARA-C and MTX; for each IVAC 2 g/m² of ARA-C, 300 mg/m² of VP-16, 1,500 mg/m² of ifosfamide and CNS prophylaxis with ARA-C and MTX. We started with the G-CSF administration at dose of 5 mg/kg at day +7 after the 2nd cycle (1st IVAC) and then we increased the dose to 10 mg/kg at day +10. At day +14 patient #1, in complete remission, had these values: WBC 4100, Hb 9.6, plt 39,000, CD34+ 4.4% that is 180.4 cells/mL. Patients #2 had WBC 6100, Hb 9.1, plt 85,000, CD34+ 1% that is 85.4 cells/mL. With this good mobilization we performed the CSC harvest by the blood cell separator COBE SPECTRA, WBC harvest set, CMN protocol using peripheral veins maintaining a constant flow of 55 mL/min. For each patient we processed 2.5 blood volumes and performed a single apheresis 190 minutes long. For patient n. 1 we harvested a total NC 33 × 10⁹, MNC 14.4 × 10⁹, CD34+ 1453.9 × 10⁶ that is 11.8 × 10⁶/kg. In the patient #2 we harvested 34.9 × 10⁹ NC, 7.9 × 10⁹ MNC, 664.6 × 10⁶ CD34+ that is 15.4 × 10⁹/kg. The CD34+ efficiency was 49.7% and 92%, respectively. We can conclude that VP-16 plus G-CSF mobilization action was very good although the very myelotoxic polychemotherapy administration, as the antimetabolites ARA-C and MTX, not only e.g. but also allowed a pharmacologic low release and then a most prolonged myelosuppressive action. This excellent mobilization allowed us to harvest about 2.5 CD34+ cell doses by a single apheresis. For the future we can single out the first IVAC as a cycle able to induce remission and to mobilize CSC at the same time.

P0343
DOSE ESCALATING OF CYCLOPHOSPHAMIDE IN THE MOBILIZATION OF CD34 CELLS IN HEMATOLOGICAL MALIGNANCIES

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The use of high dose cyclophosphamide (CTX) plus G-CSF as mobilizing agent of CD34 cells is widely acceptable in hematologic malignancies; however no standardized approach in the dose of CTX and timing of CD34 mobilization yet exists. We present our experience based on three different dose of CTX used as mobilizing therapy in patients mostly with multiple mieloma (MM), lymphomas and acute leukemia. According to the dose of CTX we recognized three groups of patients: the first group of 14 pts who received CTX at 4 g/m², 46 pts treated with 5 g/m² and 27 pts scheduled for 7 g/m². This retrospective evaluation was finalized to reveal whether if the different doses of CTX provide significant modifications of mobilization efficiency according to the number of cells collected, number of aphereses needed, time to mobilization, morbidity of CTX, need of hospitalization and time to neutropenia.
trophil recovery. Discrepancies in the distribution of disease were recorded among the groups under studies with more leukemias in the first group (50% of cases), more myelomas in the second group (41% of cases) and mostly lymphomas in the third group of patients (62% of cases); discrepancies were also recorded in the age distribution, being the mean age of 59, 61 and 38 years for the first, second and third group, respectively. The collection was successful in most of patients with 1 (7%) and 2 (5%) unsuccessful in the first and second group, respectively; the efficiency of collection was progressively better from the first to third group with 7.25, 8.49 and 14.8 × 10^5/kg median number of CD34 positive cells, respectively. Need of hospitalization or transfusion needs were recorded in 7%, 34% and 81% of patients receiving 4, 5, or 7 g/m^2 of CTX, respectively. Finally the hematological recovery after reinfusion of the collected CD34 cells was identical in the three groups of patients ranging from 8 to 14 days. We conclude that whatever the dose of CTX employed as mobilizing therapy for CD34 cells, the efficiency of collection is good in most patients, however no failures were recorded with CTX at 7 g/m^2. The incidence of side effects or need of hospitalization is the major limiting factor for using CTX at 7 g/m^2 in most of patients. The evaluation of the mobilization efficiency and the limited incidence of side effects in the group of patients treated by 5 g/m^2 of CTX give the conclusion that this approach should be recommended in most patients.

PO144
HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION IN BREAST CANCER PATIENTS WITH MORE THAN 10 POSITIVE AXILLARY NODES: PRELIMINARY RESULTS
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Purposes. To evaluate survival and sites of failure in a group of 87 breast cancer patients with massive axillary involvement (>10 positive nodes), referred to the Radiation Therapy Department of the University of Florence during the period 1994-1999. Among these patients, 46 underwent HD chemotherapy followed by autologous peripheral blood stem cell transplantation (APB-SCT) whereas 41 women underwent conventional chemotherapy. Overall survival, disease-free survival and site and frequencies of recurrences were evaluated in these patients as well. After surgery all patients underwent chemotherapy and local irradiation. Material and Methods. Forty-six patients were referred for postoperative treatment and underwent FEC chemotherapy ( 4-8 cycles) and HD chemotherapy (ifosfamide 3000 mg/m^2 on days 6-5-4-3, carboplatin 500 mg/m^2 on days 6-5-4-3 and etoposide 300 mg/m^2 on days 6-5-4-3) before autologous transplantation between 1994/1999 (GROUP A). During the same period a group of 41 patients with similar pathologic characteristics underwent conventional chemotherapy without autologous transplantation (GROUP B). The Kaplan-Meyer method was used to estimate the survival probabilities with statistical inferences on actuarial curves by using the log-rank test. The median follow-up was 44 months, with a minimum follow-up of 12. Results. Isolated local relapses occurred in only 4 in Group A and 3 in Group B. Most patients showed a distant failure in both of the groups (18/46 and 10/41 in Group A and Group B respectively). As far as concerns the number of positive axillary nodes, patients in Group A seem to show a higher number of distant metastases with the increasing number of positive nodes in the axilla. At this time, the 5-year overall survival rates were 70% and 65% for Group A and B, respectively. We observed 22 recurrences in Group A (14 distant, 4 local and 4 local and distant) and 13 in a Group B (10 distant, 3 local). No grade III or IV toxicity was present among the patients who underwent HD chemotherapy and bone marrow transplantation. Conclusions: These preliminary results show that the treatment is feasible and well tolerated; a longer follow-up period, a larger number of patients and an accurate selection of cases are needed.

PO145
AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND SOLID TUMORS: A SINGLE CENTER EXPERIENCE
Divisione Ematologia, Ospedale San Francesco ASL 3 Nuoro

Autologous stem cell transplantation (ASCT) has become a standard treatment modality for patients with hematologic malignancies and solid tumors. From August 1992 to June 2000 100 ASCT have been carried out in our center, average 12.5 per year (2-21) in 72 different patients. Nineteen of them had non Hodgkin’s lymphoma (NHL), 17 multiple myeloma (MM), 14 breast cancer (BC), 9 acute myeloid leukemia (AML), 6 Hodgkin disease (HD), 3 small cell lung cancer (SCLC), 1 acute lymphoid leukemia, 1 chronic myeloid leukemia, 1 myelodysplastic syndrome (MDS), 1 embirional carcinoma. Twenty-three patients received more than 1 transplant, 3 transplants in 5 cases (3 SCLC, 2 NHL), while 18 patients (13 MM, 3 HD, 2 BC) underwent a double transplant procedure. Patients were nursed in single room with reverse isolation, a central venous line was in place, skin tunneled in 96 cases, non-tunneled in 4 cases. Median age at transplantation was 48 years (6-73). Source of stem cells was peripheral blood in 95 cases, bone marrow (BM) in 4 cases, blood and BM in 1 case. Conditioning regimen was melphalan alone (35 cases) or combined with mitoxantrone or thiopheta and cyclophosphamide (22), BEAM (14), ICE (9), busulfan based regimens (8), other chemotherapy regimens (12). Median time to reach 500 PMN was 10 days (8-41), 20,000/µl plts 11 days (nadir at more than 20,000 in 2 cases-48 days), and 100,000/µl plts 17 days (10 days-100,000 plts not reached in 3 cases). Excluding patients with AML and MDS who did not receive G-CSF in the first 15 days and those transplanted with BM stem cells, time to 500 PMN, 20,000 plts/µl and 100,000 plts/µl was 9, 10 and 17 days respectively. No graft failure was observed. Median time as inpatients was 19 days (5-62), days with fever above 38°C ranged from 0 to 24 (median 1). Median number of red blood cell and platelets transfusions were 2 (0-8) and 1 (0-12). Median number of CD34+ cells infused was 4.3 × 10^6/kg (1-11.5). After transplantation 62% of patients were in complete remission (CR), 25% in partial remission, 11% progressed or had stable disease. Transplant related mortality was 1% (1 patient died at day +120 of fulminant hepatitis because of HBV reactivation), 33 patients
PO146  HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL  TRANSPLANTATION IN ADVANCED STAGE INTERMEDIATE-HIGH GRADE  NON-HODGKIN'S LYMPHOMAS


Hematology Service and Bone Marrow Transplantation Unit, A.O. "San G. Moscati", Avellino; *Hematology Unit, Federico II University Naples; *Divisione di Ematologia AORN A. Cardarelli, Naples; *Bone Marrow Transplantation Unit Solid Tumors Ospedale A. Cardarelli, Naples

High-dose chemotherapy (HDT) with autologous stem cell transplantation for advanced stage non-Hodgkin's lymphoma is still considered an experimental therapeutic approach and the role of transplantation in partial or complete responders is still uncertain. We report our experience of autologous stem cell transplantation (ASCT) in advanced stage non-Hodgkin's lymphoma (NHL). From March 1988 to April 2001, 47 patients (median age 38 yrs, range 5-58), with intermediate or high grade non-Hodgkin's lymphomas, 6 in stages II bulky, 21 in stage III and 20 in stage IV received unpurged ASCT after a myeloablative conditioning regimen. Twenty patients (42%) (19 NHL-HG, 1 NHL-IG) received ASCT in 1st complete remission (CR1), 8 (17%) in CR2 or CR3 (6 NHL-HG, 2 NHL-IG), 10 (21%) in partial remission (PR) (9 NHL-HG, 1 NHL-IG), 5 (11%) in chemosensitive relapse (SR) and 4 (9%) chemoresistant disease (RD). Bone marrow (BM) was used as the source of stem cells in 22 pts (47%) and peripheral blood (PB) in 25 pts (53%). As conditioning regimen 20 pts received BEAM, 15 BEAC, 8 BAVC, 4 others. G-CSF 5 mg/kg was given to 41 pts following ASCT. After BM cells median time to achieve a neutrophil count > 1×10⁹/L and a platelets count > 20×10⁹/L were 15 (range 9-21) days and 17 (range 9-35) days, respectively. After PB cells neutrophil count > 0.5×10⁹/L and a platelet count > 20×10⁹/L were reached after 10 (range 7-12) days and 22 (range 18-50) days, respectively. Four cases (18%) of transplant-related mortality (TRM) occurred in patients receiving BM versus 0% in patients receiving PB cells. After a median follow-up of 26 months (range 5-138), 17 of 20 patients (85%) transplanted in CR1 are in continuous CR. After a median follow-up of 22 mo. (range 1-55) 5 of 8 patients transplanted in CR2/C3, are in CR. Of 10 patients transplanted in PR, 5 (50%) achieved CR and 3 of these are in CR. Of 5 patients in SR, 2 achieved CR, 1 is in CR at 36 months and 1 died of lung cancer while in CR for lymphoma at 6 months, 2 had stable disease and 1 had progression. At a median follow-up of 29 months (range 1-138), DS and EFS were 71% and 66%, respectively. OS for patients in CR1, CR2/3 and PR were 83%, 73% and 50%, respectively. EFS for patients in CR1, CR2/3 and PR were 82%, 60% and 40%, respectively. By log-rank test EFS was significantly better for patients in CR1 and CR2/3 than for patients in PR (p=0.03).Our results in this small cohort of patients demonstrate the high efficacy of autologous stem cell transplantation to prolong a complete remission in patients with advanced stage NHL in CR1 and CR2/3 status at transplant but a poor efficacy in PR. The use of BM as the source resulted in higher toxicity. Large ongoing randomized studies would help to clarify the definitive role of HDT in advanced stage NHL.

PO147  AUTOLOGOUS AND ALLOGENIC TRANSPLANT IN FOLLICULAR LYMPHOMA


Division of Hematology, Department of Oncology-Hematology, Niguarda Ca’ Granda Hospital, Milan; *Department of Experimental, Environmental Medicine and Medical Biotechnologies, Milano Bicocca University

Since 1996, 15 patients (M:F = 4:11; median age 45 years; range 28 - 60) with relapsed or refractory follicular non Hodgkin’s lymphoma (WHO grade 1-2), underwent allogeneic bone marrow transplantation from HLA-identical sibling donor (2 cases) or autologous mobilized stem cell transplant (13 cases).

<table>
<thead>
<tr>
<th>Mobilization</th>
<th>Auto Tx (n=13)</th>
<th>Allo Tx (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD-CTX (n=8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DHAP (n=5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Status at Tx</td>
<td>5 RC, 8 RP</td>
<td>2 RP</td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td>BEAM (n=6)</td>
<td>L-PAM+TBI (n=7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cy+TBI</td>
</tr>
<tr>
<td>Positive CD34+ purging</td>
<td>n=7</td>
<td>0</td>
</tr>
</tbody>
</table>

Results. Transplant-related mortality (TRM) = 0/15 patients. Overall survival (OS) = 13/15 patients (86%, median survival 68 months, range 32-106). Event-free survival (EFS) = 10/15 patients (66%, median 22 months): 3 patients showed progression or relapse (+13, +19, +45 months post-ABMT); two patients died of disease progression at +12 and +21 months after ASCT; both the allogeneic transplanted patients are alive and disease free at +29 and +44 months from BMT. A molecular study (PCR) demonstrated the presence of t(14;18) (11 patients) or the IgH rearrangement (3 cases) before transplant; monitoring by PCR is ongoing during the follow-up period (every 3-6 months post transplant). Conclusions. The molecular quantitative analysis of t(14;18)-positive cells before and after autologous and allogeneic transplant is on process in order to evaluate the molecular eradication and the relapsing time.
supportive therapy and techniques

PO348
CYTOMEGALOVIRUS ENCEPHALITIS IN A PATIENT TRANSPLANTED WITH T-CELL DEPLETED HAPLOIDENTICAL PERIPHERAL BLOOD STEM CELLS: CASE REPORT

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Hematology, University "Tor Vergata", St. Eugenio Hospital, Rome

In December 97 a 20-year-old male presented with a diagnosis of severe aplastic anemia. He was treated with a course of immunosuppressive therapy (steroids, cyclosporine, SAL and G-CSF). Response was obtained after 90 days. One year later he relapsed; since an HLA-identical family was not available, a T-cell depleted haploidentical PBSCT was performed from his mother according to the Perugia protocol. Nested PCR and pp65 antigenemia were used to monitor CMV infection, weekly. He was treated with foscarnet+ganciclovir and specific immunoglobulins when CMV pp65 antigenemia was detected in peripheral blood cells. Therapy was maintained for seven months. He recovered T-cell immunity in these patients, who show a slow T-cell recovery with persistent immunosuppression.

On day +60 CMV pp65 antigenemia was detected in peripheral blood cells and antiviral prophylaxis was given according to the Perugia protocol. Nested PCR and pp65 antigenemia were used to monitor CMV infection, weekly. He was treated with foscarnet+ganciclovir and specific immunoglobulins when CMV pp65 antigenemia was detected in peripheral blood cells. Therapy was maintained for seven months. He recovered T-cell immunity in these patients, who show a slow T-cell recovery with persistent immunosuppression.

PO349
GENETIC SCREENING FOR HEMOCROMATOSIS IN ITALIAN PROSPECTIVE BLOOD DONORS WITH IRON OVERLOAD

Blood Transfusion Centers: 1Sondrio, 2Bolzano, 3Savigliano, 4Rovigo, 5 Udine, 6 Fabriano, 7 Bari, 8 Ragusa; 1Department of Internal Medicine, University and Ospedale Maggiore Policlinico IRCCS, Milano

Objectives. To analyze the role of HFE mutations in Italian blood donors with iron parameters suggesting iron overload.

Methods. Screening was based on transferrin saturation (TS) and serum ferritin (SF) (TS>50% and >45; SF>300 and >250 μg/mL in males and females respectively) in 5880 subjects (3640 males, 2240 females, age range 18-60) undergoing evaluation for blood donation eligibility, originating from different areas of Italy (north: 4058; south: 1822). Subjects with increased TS and/or SF were re-tested and typed for HFE mutations C282Y and H63D. Results. Overall, 179/548 subjects with abnormal parameters were available for re-testing, and in 109 increased TS and/or SF were confirmed. Increased TS was confirmed in 25 individuals among whom 3 were C282Y homozygotes and 6 were compound heterozygotes for C282Y and H63D. In these 9 subjects ferritin was increased in 6 and normal in 3 cases. Isolated increased SF was confirmed in 84 individuals. Among them we found an increased frequency of mutations as compared with the normal Italian population (C282Y 0.06; H63D 0.25), as well as a high frequency (45%) of increased alcohol intake. High TS was more frequent in northern-Italy than in southern regions (p=0.02), while the geographical distribution of high SF was not significantly different. In individuals with increased TS and/or SF the frequency of C282Y and H63D was 0.13 and 0.21 in northern-Italy vs. 0.05 and 0.45 in southern-Italy (p=0.004 for H63D). Nine out of 10 individuals carrying hemochromatosis-associated genotypes (homozygosity for C282Y and compound het-

PO150
SEVERE INFECTIONS RISK FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA

A. Molteni, A. Nosari, P. Bemuzzi, L. Gargantini, E. Pungolino, L. Barbarano, M. Montillo, E. Morra
Division of Hematology, Dept. Oncology-Hematology, Niguarda Ca’ Granda Hospital, Milan

Infections are the major cause of morbidity and mortality in patients with chronic lymphocytic leukemia (CLL). As well as the immune defects inherent to the disease itself and its management, hypogammaglobulinemia is considered an important risk factor. Newer treatments such as purine analogs may be associated with a new spectrum of pathogens involving T cell dys-

Methods. Screening was based on transferrin saturation (TS) and serum ferritin (SF) (TS>50% and >45; SF>300 and >250 μg/mL in males and females respectively) in 5880 subjects (3640 males, 2240 females, age range 18-60) undergoing evaluation for blood donation eligibility, originating from different areas of Italy (north: 4058; south: 1822). Subjects with increased TS and/or SF were re-tested and typed for HFE mutations C282Y and H63D. Results. Overall, 179/548 subjects with abnormal parameters were available for re-testing, and in 109 increased TS and/or SF were confirmed. Increased TS was confirmed in 25 individuals among whom 3 were C282Y homozygotes and 6 were compound heterozygotes for C282Y and H63D. In these 9 subjects ferritin was increased in 6 and normal in 3 cases. Isolated increased SF was confirmed in 84 individuals. Among them we found an increased frequency of mutations as compared with the normal Italian population (C282Y 0.06; H63D 0.25), as well as a high frequency (45%) of increased alcohol intake. High TS was more frequent in northern-Italy than in southern regions (p=0.02), while the geographical distribution of high SF was not significantly different. In individuals with increased TS and/or SF the frequency of C282Y and H63D was 0.13 and 0.21 in northern-Italy vs. 0.05 and 0.45 in southern-Italy (p=0.004 for H63D). Nine out of 10 individuals carrying hemochromatosis-associated genotypes (homozygosity for C282Y and compound het-

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eosinophilic | March 16, 2001 | Volume 36 | Issue 3 | Page 451

The infections registered are detailed in Table 1. Documented moderate infections were especially represented by bronchitis (61 cases), genitourinary infections (23 cases) and herpetic infections (herpes simplex in 14 cases, herpes zoster in 24 cases). Among documented severe infections the most frequent were pneumonia (48 cases).
Among severe infections we observed two cases of multifocal progressive leukoencephalopathy. We considered the incidence of infections in accordance to risk factors and the prevalence of severe infections in each risk category (Table 2).  

Table 1.  

<table>
<thead>
<tr>
<th>Total number of patients</th>
<th>333 (M187, F146)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with infection</td>
<td>106 (31.8%)</td>
</tr>
<tr>
<td>Number of febrile episodes</td>
<td></td>
</tr>
<tr>
<td>F/U/O</td>
<td>70 (26%)</td>
</tr>
<tr>
<td>Moderate infection</td>
<td>144 (53%)</td>
</tr>
<tr>
<td>Severe infection</td>
<td>59 (21%)</td>
</tr>
<tr>
<td>Documented infections</td>
<td></td>
</tr>
<tr>
<td>Bacterial</td>
<td>147 (72%)</td>
</tr>
<tr>
<td>Viral</td>
<td>40 (19%)</td>
</tr>
<tr>
<td>Fungi, protozoa, mycobact.</td>
<td>16 (7%)</td>
</tr>
</tbody>
</table>

Table 2.  

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Incidence of infections</th>
<th>Prevalence of severe infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>H ypogammaglobulinemia</td>
<td>61%</td>
<td>20.1%</td>
</tr>
<tr>
<td>Treatment with fludarabine</td>
<td>77%</td>
<td>16.6%</td>
</tr>
<tr>
<td>Treatment with more than two chemotherapy lines</td>
<td>83%</td>
<td>38%</td>
</tr>
<tr>
<td>Binet Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>21%</td>
<td>17%</td>
</tr>
<tr>
<td>B</td>
<td>40%</td>
<td>21%</td>
</tr>
<tr>
<td>C</td>
<td>51%</td>
<td>24%</td>
</tr>
</tbody>
</table>

We did not observe a correlation between age and severity of infections: as a matter of fact average and median age were respectively 68 and 70 in patients with severe infections, while they were respectively 66 and 65 in patients with moderate ones. Conclusions. The most important risk factor in CLL is a sec-

PO151  
LAMIDUVINE AS PRIMARY PROPHYLAXIS OF CHEMOTHERAPY-INDUCED HEPATITIS B VIRUS REACTIVATION IN HBsAG-POSITIVE PATIENTS WITH HEMATOLOGIC MALIGNANCI ES  
A.M. Pelizzari, M. Motta, G. Rossi  
Sezione Ematologia, Dipartimento di Medicina, Spedali Civili, Brescia  
Cancer chemotherapy (CT) in chronic carriers of hepatitis B virus (HBV) can promote viral replication, and after immunosuppressive treatment is stopped, the return of immune competence can frequently be followed by severe liver damage, including fulminant hepatitis. In a retrospective analysis of a 20-month period at our center, 6 acute HBV reactivations were recorded in patients (pts) with hematologic malignancies treated with CT, a median of one month after the last cycle. Three pts died with acute hepatic failure, chronic aggressive hepatitis developed in one. CT was stopped in 2/3 surviving pts. The frequency of HBV reactivation among all pts with non-Hodgkin’s lymphoma (NHL) treated at our Institution during the same period, was 43% (3/7) in HBsAg-positive pts compared to 0% in HBsAg-negative pts (0/49) (p=0.0013) and in pts with unknown HBV status (0/25) (p=0.0071). Lamivudine can directly suppress HBV replication, is well tolerated and causes few hematologic side effects. It has been recently used both in the treatment and in the secondary prevention of CT-induced HBV reactivation. Since August 1999 we have used lamivudine as primary prophylaxis of HBV reactivation in 34 consecutive HBsAg-positive patients with hematologic malignancies treated with CT at our Institution. Their median age was 61 (range 29-80). Lamivudine was given orally at the dose of 100 mg daily from the start until one month after the end of CT. Hematologic disorders included low-grade (11) and high-grade NHL (11), chronic lymphatic leukemia (7), acute myeloid leukemia (2), others (3). The CT programs used were CHOP (8), ACVBP (4), fludarabine/2CDA+alkylating agents (10), alkylating agents+prednisone (6), HD-cyclophosphamide (2) and others (4). Before treatment 9 pts (26%) had elevated transaminase levels, 11 (32%) had detectable serum HBV-DNA, 6 (18%) were HbsAg-positive. None showed signs of liver failure. Lamivudine treatment was well tolerated. It was transiently withdrawn in one patient because of a suspected allergic reaction. A self-limiting 3-fold increase of serum amylase was observed in 1 case. HBV-DNA levels decreased in all cases (p=0.0389) and transaminase levels in 8/9 cases (p=0.057). In one patient HBsAg became undetectable. One patient developed transient hepato-
sis during treatment (HBV-DNA: 29 U/l) and CT was resumed upon recovery without sequelae. HBV reactivation with mild hepatitis occurred in 3 patients. 1-3 months after lamivudine withdrawal. In the other 30 pts, no signs of HBV reactivation nor of transaminase increase was detected both during and after treatment, with a median follow-up of 6 months (range 1-16). All patients could complete their planned chemotherapy programs with reductions of cytostatic drug dosages and results comparable to those expected in HBsAg-negative patients. We conclude that HBV reactivation is a frequent and potentially dangerous complication in HBsAg-positive pts treated with CT for hematologic malignancies and that primary prophylaxis with lamivudine given in association with CT is a well tolerated and effective method for its prevention.

PO152  
CIRCULATING GALACTOMANNAN SCREENING IN STEM CELL TRANSPLANT RECIPIENTS FOR DIAGNOSIS OF INVASIVE ASPERGILLOSIS  
M. Cuzzola, G. Irrera, O. Iacopino, A. Cuzzocrea, F. Morabito, P. Iacopino  
Centro Unico Regionale Trapianti di Midollo Osseo, A.O. Bianchi-Melacrino, Morelli, Reggio Calabria  
The diagnosis of invasive aspergillosis (IA) has mainly been approached either estimating the disease probability by clinical and radiological criteria or by microbiological and histological approaches, which usually require invasive procedures with unacceptable delay of therapy. More recently, a method for early IA diagnosis has been validated in hematological patients at high risk of developing IA. In our study, 33 patients with hematologic malignancies (30 cases) or solid tumors were prospectively evaluated for galactomannan (GM) positivity (Elisa assay,
with fludarabine 30 mg/m² on day –4, –3 and –2, and low-dose plant from an HLA identical sibling. The patient was conditioned non-myeloablative allogeneic peripheral blood stem cell transplant after two chemotherapy induction treatment underwent a di Pisa U.O. Ematologia, Università degli Studi di Pisa e Azienda ALLOGENEIC STEM CELL TRANSPLANT FATAL HERPESVIRUS 6 ENCEPHALITIS FOLLOWING A NON-MYELOABLATIVE approach to prophylaxis and therapy of aspergillosis. died, including 2 patients with a possible and 2 patients with a positive GM became ambisome. Six of the 11 GM-positive cases died, including 2 patients with a possible and 2 patients with a positive GM showed positivity, while 50% (3/6) of the previously negative cases became GM-positive. Of these positive cases, 3 cases started and 4 continued treatment with ambisome, while the remaining maintained itraconazole. Six of the 11 GM-positive cases died, including 2 patients with a possible and 2 patients with a probable IA; of note, all of them were on ambisome therapy. In conclusion, GM determination may be useful for an early approach to prophylaxis and therapy of aspergillosis.

Supported in part by AIL and Regione Calabria.

POI53 FATAL HERPESVIRUS 6 ENCEPHALITIS FOLLOWING A NON-MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANT

E. Benedetti, S. Galimberti, L. Ceccherini,* F. Caraccio, R. Riccioni, F. Papineschi
U.O. Ematologia, Università degli Studi di Pisa e Azienda Ospedaliera Pisana; *U.O. di Microbiologia Università degli Studi di Pisa

A 67-year old female with a diagnosis of acute myelogenous leukemia standard risk by cytogenetics, in 1st complete remission after two chemotherapy induction treatment underwent a non-myeloablative allogeneic peripheral blood stem cell transplant from an HLA identical sibling. The patient was conditioned with fludarabine 30 mg/m² on day –4, –3 and –2, and low-dose TBI (2000CyG) and postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSA). MMF was discontinued by day 27. Day +28 assessment for donor chimerism showed: donor T cells 90%; granulocytes 100%; bone marrow 95%. Bone marrow evaluation showed complete remission by morphology and flow cytometry. By day +31 the patient experienced grade II aGVHD (skin and upper gastrointestinal tract) and methylprednisolone was added to cyclosporine. Suddenly, at day +35 the patient appeared lethargic but without neurological focal signs. The day after, cyclosporine was discontinued in the suspicion of cyclosporine toxicity, a CT scan was performed which showed no focal lesions, neurological assessment was again negative for focal signs and a lumbar puncture was performed. Extensive virological and microbiological assay of cerebrospinal fluid allowed the identification of HHV6 DNA. The patient became progressively more lethargic and died two days later. This case illustrates the importance of considering HHV6 as a possible pathogen also in the setting of non-myeloablative allogeneic peripheral blood stem cell transplant.

POI54 FEBRILE NEUTROPIA IN HEMATOLOGICAL PATIENTS. WHICH ROLE FOR AN OUTPATIENT MANAGEMENT?

F. Rossini, I. Miccolis, M. Fumagalli, E. Manca, M. Verga, E.M. Pogliani, G. Comeo
Hematology Unit, University of Milan, Monza

Among patients developing febrile neutropenia while at home, some are treated as outpatients, generally with oral therapy, while some are admitted to the hospital and treated with parenteral antibacterial therapy. This decision is based on the evaluation of different prognostic factors. The aim of this study was to evaluate characteristics, risk factors and outcome of high risk patients who are treated as inpatients, trying to identify a subgroup of patients who could be candidate for outpatient management. Patients and Methods. During 1999 and 2000, 145 patients were admitted because of febrile neutropenia. They were affected by: acute myelogenous leukemia, 36; acute lymphoblastic leukemia, 13; chronic myeloid leukemia, 7; chronic lymphocytic leukemia, 30; Hodgkin’s disease, 5; non-Hodgkin’s lymphoma, 32; myeloma, 22. Most of them had uncontrolled cancer. They represented 14% of admissions in adult patients affected by these diseases in the whole hospital. Median age was 61.4; median duration of hospital stay was 18.1 days. All patients received a combination of amikacin and a cephalosporin; in most cases ceftriaxone was used with a once-a-day antibiotic therapy; antifungal therapy was added when indicated. Results and conclusions: when the 3 patients with septic shock were excluded (1 of them died), we have divided the patients in two risk groups, according to the presence of pneumonia. The first group was composed by 68 patients who had pneumonia (mean age: 64.7, mean duration of hospital stay: 19 days); 21 (30.8%) of them died, after a mean of 9.8 days. Eleven of 21 died within one week; ten had uncontrolled cancer and pneumonia represented the terminal event. We have considered the remaining 74 patients in a low risk group. They represented 7% of admissions, (mean age 58.5, mean duration of hospital stay 17.3 days); 14 (18.9%) of them died, after a mean of 23.3 days. Three of them died within one week, all with uncontrolled cancer. The death rate was significantly lower (p=0.05) than in the previous group. Our data confirm that the prognosis of this group of patients remains poor and that uncontrolled cancer can account for a significant percentage of mortality. However, even if the situation of any single patient must be daily and carefully considered, we can conclude that an outpatient management can be initially proposed in a significant group of neutropenic febrile patients, utilizing once-a-day antibiotic therapy.

POI55 HEMATOLOGIC HOME CARE UNIT: PRELIMINARY RESULTS OF A SINGLE CENTER EXPERIENCE

B. Ronci, L. Spila, E. Piro, M. Persiani, A. Fremiotti
U.O.D. Azienda Ospedaliera S. Giovanni-Addolorata, Rome

On the first of October 2000, authorized by the Assessatore alla Sanità della Regione Lazio, our institution started the Ospedale Domiciliare Ematologico (O.D.E.) (Hematologic home care unit). By home care unit, in accordance with many region-
al and national regulations, we aimed to perform, at the patients’ home, diagnostic and therapeutic acts which are normally performed by traditional hospital admission. In particular the O.D.E. works on selected hematological patients, admitted to the Hematological ward of S. Giovanni-Addolorata Hospital, the medical chief is a hematologist in order at the Hematological ward and strictly connected at this Institution. In the first six months of activity the O.D.E. developed treatment in three categories of hematological patients: 1) advanced disease or short term poor prognosis patients. These patients are not the main target of O.D.E. activity because they need less specific therapy and for whom we think useful others kind of home care such as integrated home care. Less than 10 percent of O.D.E. patients are included in this category 2) patients introduced in a diagnostic plan and or an initial treatment in traditional hospitalisation, for physical or psychosocial reasons cannot to be admitted at the Day Hospital, performed their therapeutic plan with the O.D.E. This category included 56% of all O.D.E. patients; they are affected by chronic myeloproliferative syndromes (25 %), multiple myeloma (20 %), lymphoma (9 %), others pathologies (4 %). We believe that these patients’ category are the most suitable for hematology home care. We consider: a) fast reinstatement in own family life and improvement of patient quality-life, b) more involvement of relatives who attend to the patient at home, c) qualitatively and quantitatively improved management of the traditional hospital 3) elderly patients (> 80 years old) affected by hematological diseases who need, essentially, transfusion supportive care. This category there are about 35% of O.D.E. patient. They are patients affected by poor prognosis myelodysplastic syndromes (32 %) or oligoblastic acute myeloid leukemia (10 %). This single centre experience would demonstrate continuity between the hospital and the patient home with results of qualified cure according to human and psychological points of view.

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INFECTIOUS COMPLICATIONS IN MULTIPLE MYELOMA PATIENTS RECEIVING VINCRISTINE-ADRIAMYCIN-DEXAMETHASONE 
COMBINATION CHEMOTHERAPY

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Vinoreistine-adriamycin- dexamethasone (VAD) chemotherapy (CT) induces rapid and marked responses in both newly diagnosed multiple myeloma (MM) and relapsed or refractory disease. At present, VAD is the standard debulking (CT) in MM undergoing stem cell transplantation procedures. VAD-related infections are frequently reported and may delay the planned treatment program in single cases. We evaluated the incidence of infections and assessed factors eventually associated with a higher risk of such complications in VAD-treated patients. Forty-five patients receiving a total of 176 VAD cycles (continuous infusion [CI] schedule [vinoreistine 0.4 mg/m² c.i. days 1-4, adriamycin 9 mg/m² c.i. days 1-4, dexamethasone 40 mg i.v. days 1-4, 9-12, 17-20]; n=110, and push schedule [vinoreistine 2 mg i.v. day 1, adriamycin 50 mg/m² i.v. day 1, dexamethasone 40 mg i.m. days 1-4, 15-18]; n=66) were included in the study. The univariate Cox model was used to identify factors associated with an increased tendency to develop infections during VAD administration. There were 41 newly diagnosed MM, and 4 refractory or relapsed MM. Nine, 2, 22 and 11 patients had stage IA, IIA, IIIA and IIIB MM, respectively. Twenty-five, 8 and 11 patients had IgG, IgA and urinary light chain disease, respectively. One patient had extra-medullary plasmacytoma. Patients received a median of 4 VAD cycles (range, 2-6). In 16 cases, CT was infused via a central venous catheter (CVC). Prophylactic antibiotics and antifungal agents were not routinely given. Severe infections occurred in 10 (9.1%) and 3 (4.6%) patients during the CI and the push schedule, respectively; moderate infections were registered in 7 (6.4%) and 6 (9.1%) patients during the CI and the push schedule, respectively:

<table>
<thead>
<tr>
<th>Type of Infection</th>
<th>No. of patients (%)</th>
<th>Severe entity</th>
<th>Moderate entity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever of Unknown Origin (FUO)</td>
<td>1 (0.6)</td>
<td>7 (4)</td>
<td></td>
</tr>
<tr>
<td>Bronchopneumonia Disease</td>
<td>10 (5.7)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Urinary Tract</td>
<td>1 (0.6)</td>
<td>3 (1.7)</td>
<td></td>
</tr>
<tr>
<td>CVC Exit-Point</td>
<td>1 (0.6)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ora Candidosis</td>
<td>-</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>Cutaenous Herpes Simplex</td>
<td>-</td>
<td>1 (0.6)</td>
<td></td>
</tr>
</tbody>
</table>

Neither invasive fungal infection nor infective death was registered. In one case pneumonia was associated with Pseudomonas aeruginosa septicemia. Both FUO and pneumonias responded to empiric antibiotics. At univariate analysis, a neutrophil count < 1000/mm³ at nadir was significantly related with an increased risk of infection development. Age, sex, type of paraprotein, stage, number of previous CT cycles, reduction of polyclonal immunoglobulins, schedule and the presence of a CVC did not influence this complication. Antibiotic prophylaxis could be effective in patients presenting neutropenia during the first CT cycle.

PO157
RETROSPECTIVE ANALYSIS OF CENTRAL VENOUS CATHER-RELATED COMPLICATIONS IN A COHORT OF 126 ONCOHEMATOLOGIC PATIENTS

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Most hospitalized patients with oncohematologic disease undergo insertion of a central venous catheter (CVC), allowing increased ease and safety of administration of chemotherapy, parenteral nutrition and blood components, blood sampling and vital parameter monitoring. CVCS however induce or enhance complications, either thrombotic or infectious; the optimal prophylaxis of which is still matter of debate. In particular, while there are plenty of studies on ICU or solid cancer patients, few data are available on large series of oncohematologic patients, characterized by profound and long-lasting neutropenia and thrombocytopenia. Therefore, we retrospectively analyzed the incidence of these CVC-related complications in a cohort of oncohematologic patients consecutively hospitalized at our...
44 scored positive for gram+ agents, 2 gram-, 1 yeast. BSI
occurred in 47 of 150 (31%) evaluated CVCs. Of these,
CICC; 4. a prevalence of CR-BSI of 19.5% (16/82); 5. a prevalence
higher incidence of thrombotic complications in PICC than
undergoing LMWH prophylaxis as compared with UFH c.i.; 3. a
observed a decrease in thrombotic complications in patients
prevalence of CR-BSI of 19.5% (16/82). In conclusion: 1. The
ing agent was concordant with the blood isolated, giving a
occurred in 26 of the colonized CVCs; in 16 cases the coloniz-
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M.C. Bertoncelli,* G. Zaccala,* C. Gigli,° G. Massara#
F. Bobbio,* P. Zigrossi,* L. Paccagnino,* M. Dugnani*
PO158
was isolated: 66 gram+, 7 gram–, 1 yeast, 5 mixed gram+/gram–,
cultures were performed. In 82 (56%) cases a BSI causative agent
No hemorragic complications were observed. We documented
prophylaxis was associated with a 19% (3/16) complication rate.
ly more frequent in pts undergoing prophylaxis with UFH than
17%) CICCs. Interestingly these complications were significant-
26%), than with non tunneled (14/126; 11%), or tunneled (2/12;
17%) CICCs. Complications were more frequently seen with PICCs (18/70;
26%), than with non tunneled (14/126; 11%), or tunneled (2/12;
17%) CICCs. Interestingly these complications were significant-
more frequent in pts undergoing prophylaxis with UFH than
LMWH: 30/170 (18%) vs 0/20 CVCs (chi square test: p=0.04). No prophylaxis was associated with a 19% (3/16) complication rate.
No hemorrhagic complications were observed. We documented
147 febrile episodes with clinical evidence of sepsis and blood
cultures were performed. In 82 (56%) cases a BSI causative agent
was isolated: 66 gram-, 7 gram+, 1 yeast, 5 mixed gram+/gram–,
2 mixed gram+/yeasts, 1 mixed anaerobic/gram- . CVC tip coloni-
zation occurred in 45 of 150 (31%) evaluated CVCs. Of these,
44 scored positive for gram+ agents, 2 gram-, 1 yeast. BSI
occurred in 26 of the colonized CVCs; in 16 cases the coloniz-
ing agent was concordant with the blood isolated, giving a
prevalence of CR-BSI of 19.5% (16/82). In conclusion: 1. The
incidence of thrombotic complications in our series is compara-
ble with that described in non trombocitopenic patients; 2. we
observed a decrease in thrombotic complications in patients
undergoing LMWH prophylaxis as compared with UFH c.i.; 3. a
higher incidence of thrombotic complications in PICC than in
CICC; 4. a prevalence of CR-BSI of 19.5% (16/82); 5. a prevalence of Gram- coci as causative agents of catheter colonization,
bacteremia and BSI and CR-BSI.

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LIPOSMAL AMPHOTERICIN B (AMBISOME) IN THE PROPHYLAXIS OF
INVASIVE FUNGAL INFECTIONS IN BONE MARROW TRANSPLANT RECIPIENTS
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Invasive fungal infections in immunosuppressed bone marrow recipients are often fatal and may benefit from a preventive approach. Until recently, one of the few antifungal agents with activity against both Candida and Aspergillus was amphotericin B. However, amphotericin B is commonly associated with adverse effects and toxicity. Various liposomal encapsulations of amphotericin B as well as Ambisome have been shown to decrease toxicity while maintaining or even enhancing the therapeutic efficacy. The objective of our study was to determine the efficacy and safety of Ambisome as prophylaxis of fungal infections in allogeneic and autologous BMT recipients. Sixty-one adult patients (34 male and 27 female with median age 43), with hematologic malignancies and treated with allogeneic (n=16) or autologous (n=45) blood/marrow transplantation, received 1 mg/kg/day of Ambisome as prophylactic treatment. The drug was started when neutrophil count had decreased to < 0.5 x 10^9/L and was continued until neutrophils recovered to this level or an infection or toxicity end-point was reached. Prophylaxis did not exceed 3 months. At the first sign of infection, broad-spectrum

institution from Jan. 1999 to Jan. 2001. One-hundred and twenty-six patients (75 M/56 F, median age 54 yrs, range 15–83,
acute leukemia 83, lymphoproliferative disorders 29, aplastic
anaemia 7, myelodysplastic disorders 3, others 2) underwent the
implantation of 208 CVCs: 138 centrally inserted subclavian,
CICCs, (126 non tunneled, 12 tunneled). 70 peripherally insert-
ed CVCs, PICCs. The median duration of catheterization was 20
days (range 1–98), for a total of 4051 catheter/days. Antithrom-
botic prophylaxis was as follows: unfractionated heparin (UFH)
2500 IU c.i. (170 CVCs), low molecular weight heparin (LMWH)
4000 IU i.v. single bolus (20 CVCs), other (2 CVCs); in 16 CVCs
no prophylaxis was administered. Our standard antibiotic pro-
phylaxis was daily levofloxacin 500 mg po. Thrombotic compli-
cations developed in 34/208 (16.3 %) CVCs i.e. 1/119 cath./day.
In particular deep vein thrombosis (DVT) occurred in 6 (3%),
thrombophlebitis (TF) in 11 (5%), CVC malfunctioning in 17 (8%).
Complications were more frequently seen with PICCs (18/70;
26%), than with non tunneled (14/126; 11%), or tunneled (2/12;
17%) CICCs. Interestingly these complications were significant-
ly more frequent in pts undergoing prophylaxis with UFH than
LMWH: 30/170 (18%) vs 0/20 CVCs (chi square test: p=0.04). No prophylaxis was associated with a 19% (3/16) complication rate.
No hemorrhagic complications were observed. We documented
147 febrile episodes with clinical evidence of sepsis and blood
cultures were performed. In 82 (56%) cases a BSI causative agent
was isolated: 66 gram-, 7 gram+, 1 yeast, 5 mixed gram+/gram–,
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CICC; 4. a prevalence of CR-BSI of 19.5% (16/82); 5. a prevalence of Gram- coci as causative agents of catheter colonization,
bacteremia and BSI and CR-BSI.

PO158
STRONGYLOIDES HYPERINFECTION SYNDROME: A DANGER FROM THE PAST

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Strongyloides stercoralis can cause systemic and disseminated infection in immunocompromised individuals. The immunocompetent subject strongyloidiasis can be completely asymptomatic and often persists for tens of years given the characteristic life-cycle of this nematode. The beginning of the infection is often hidden in the past and the worm can show itself only when the T-cell mediated immunity is compromised. S. stercoralis is a world-wide infection both in developed and in developing countries although with quite different incidences and prevalences. It is difficult to diagnose the infection in immunocompetent subjects: the copro- parasitological test, often underestimated, has a very low sensitivity. The only clinical sign is a mild eosinophilia that can be variable and in the last 2 years we have observed this syndrome in four hematological patients. The first one with B-chronic lymphocytic leukemia (CLL) showed S. stercoralis infection as a terminal event of seven years of disease. The second one with multiple myeloma suffered from vomiting and abdominal pain after the third cycle of chemotherapy (CT). The histologic specimen of duodenal epithelium showed S. stercoralis larvae and after 2 cycles of 7 day-therapy with albendazole we observed the normalization of the copro-parasitological test. The third case is a patient suffering from CLL for 3 years who after the 4th cycle of CT with fludarabine, mitoxantrone and dexamethasone showed severe eosinophilia with parasitological test positive for larvae of S. stercoralis. He was treated successfully with albendazole for 7 days, but the eosinophilia turned to normal only after a second cycle of therapy with albendazole. The last patient is a woman treated for non-Hodgkin’s lymphoma (NHL) with many episodes of septicaemia also after the resolution of the aplastic post-CT period. The patient was treated with granulocyte growth factor, antibiotics and antimycotics without a complete remission of clinical symptoms. Further stool checks found numerous larvae of S. stercoralis. First the patient was given albendazole for 10 days, but the larvae in the stool disappeared only after orally ivermectine, with clinical improvement of the patient. There are many case-reports of Strongyloides hyperinfection in hematological malignancies, transplants, rheumatological diseases, etc. consequently it must be remembered that this nematode can cause potentially fatal infection which can however be cured if diagnosed early. We stress the clinical importance of the copro-parasitological test in every immunocompromised patient with septicemia.

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other fungi are recognized as the cause of disseminated or local malignancies. Among fungal species, notwithstanding the cause of morbidity and mortality in patients with hematologi—py and supportive care, fungal infections remain a significant problem with a higher incidence in immunocompromised hosts. The use of antifungal prophylaxis has been recommended for patients at risk of developing fungal infections. However, the safety and efficacy of antifungal prophylaxis remain under investigation. Despite recent advances in antimicrobial therapy and supportive care, fungal infections remain a significant cause of morbidity and mortality in patients with hematological malignancies. Among fungal species, notwithstanding the prevalence of Candida and Aspergillus, an increasing number of other fungi are recognized as the cause of disseminated or localized infection in immunocompromised hosts. Fusarium is a ubiquitous fungus, disseminated worldwide and generally found in the soil and in plants. Fusarium solani is the most common species. Disseminated fusariosis in immunodeficient patients results from infection through the respiratory and gastrointestinal tract, from the damaged skin barrier or central venous catheters and may result in multiple-organ involvement, skin and ocular lesions. We present the case of a disseminated fusariosis with skin and ocular lesions in a patient with a resistant acute myeloid leukemia. Case report. A 31-year-old man was diagnosed as having acute myeloid leukemia in November 2000. The patient's medical history was not significant. He received two courses of chemotherapy (the first including fludarabine, cytarabine and idarubicin and the second with high-dose cytarabine) without obtaining a complete hematologic response. In January 2001 he received a third course with mitoxantrone, cytarabine and etooside. During the neutropenic phase he developed fever and intense myalgias of the legs; few days later numerous purple-red painful nodules appeared on both lower and upper limbs and the abdomen. A skin biopsy showed numerous hyphal elements, microbiologically diagnosed to be Fusarium solani. He was treated with liposomal amphotericin B (Ambisome) 3 mg/kg/die, with a progressive improvement of the clinical conditions and disappearance of the skin lesions. After 3 weeks, in which antifungal therapy was continued at the same dose, the patient developed the clinical picture of bilateral endophthalmitis with retinal involvement and rapid visual loss that progressed to complete bilateral blindness. The cytological and microbiological analysis of aqueous and vitreous fluids revealed the presence of numerous elongated fungal filaments, later confirmed to be Fusarium solani. Liposomal amphotericin B daily dose was further increased (up to 5 mg/kg) and a bilateral vitrectomy was performed. Despite this intensive treatment to date the clinical condition remains unchanged, without any regain of visual acuity. Conclusions. Fusarium solani is a rare cause of disseminated infection in immunocompromised patients. Up to date, sporadic cases of infections due to Fusarium spp. have been described; even rarer have been the reports of ocular involvement, and in these cases the clinical course has often been unfavorable. The case we present confirms the difficulties in eradicating the Fusarium infection despite the use of high dose systemic antifungals (liposomal Amphotericin B) and surgical therapy.

**PO161**

ENDOGENOUS ENDOPHTHALMITIS FOLLOWING DISSEMINATED FUNGEMIA DUE TO FUSSARIUM SOLANI IN AN ACUTE MYELOID LEUKEMIA PATIENT: A CASE REPORT

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Chair and Division of Hematology, University of Udine

Introduction. Despite recent advances in antimicrobial therapy and supportive care, fungal infections remain a significant cause of morbidity and mortality in patients with hematological malignancies. Among fungal species, notwithstanding the prevalence of Candida and Aspergillus, an increasing number of other fungi are recognized as the cause of disseminated or localized infection in immunocompromised hosts. Fusarium is a ubiquitous fungus, disseminated worldwide and generally found in the soil and in plants. Fusarium solani is the most common species. Disseminated fusariosis in immunodeficient patients results from infection through the respiratory and gastrointestinal tract, from the damaged skin barrier or central venous catheters and may result in multiple-organ involvement, skin and ocular lesions. We present the case of a disseminated fusariosis with skin and ocular lesions in a patient with a resistant acute myeloid leukemia. Case report. A 31-year-old man was diagnosed as having acute myeloid leukemia in November 2000. The patient's medical history was not significant. He received two courses of chemotherapy (the first including fludarabine, cytarabine and idarubicin and the second with high-dose cytarabine) without obtaining a complete hematologic response. In January 2001 he received a third course with mitoxantrone, cytarabine and etooside. During the neutropenic phase he developed fever and intense myalgias of the legs; few days later numerous purple-red painful nodules appeared on both lower and upper limbs and the abdomen. A skin biopsy showed numerous hyphal elements, microbiologically diagnosed to be Fusarium solani. He was treated with liposomal amphotericin B (Ambisome) 3 mg/kg/die, with a progressive improvement of the clinical conditions and disappearance of the skin lesions. After 3 weeks, in which antifungal therapy was continued at the same dose, the patient developed the clinical picture of bilateral endophthalmitis with retinal involvement and rapid visual loss that progressed to complete bilateral blindness. The cytological and microbiological analysis of aqueous and vitreous fluids revealed the presence of numerous elongated fungal filaments, later confirmed to be Fusarium solani. Liposomal amphotericin B daily dose was further increased (up to 5 mg/kg) and a bilateral vitrectomy was performed. Despite this intensive treatment to date the clinical condition remains unchanged, without any regain of visual acuity. Conclusions. Fusarium solani is a rare cause of disseminated infection in immunocompromised patients. Up to date, sporadic cases of infections due to Fusarium spp. have been described; even rarer have been the reports of ocular involvement, and in these cases the clinical course has often been unfavorable. The case we present confirms the difficulties in eradicating the Fusarium infection despite the use of high dose systemic antifungals (liposomal Amphotericin B) and surgical therapy.

**PO161**

A NEW FEASIBLE, SAFE ECHOGUIDED APPROACH FOR CENTRAL VENOUS CATHETER INSERTION IN ONCO-HEMATOLOGICAL PATIENTS

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The employment of a central venous catheter (CVC) constitutes a matter of debate concerning different routes of insertion, permanent or untunneled catheters, days in situ, etc. We evaluated feasibility and safety of a new procedure of CVC insertion in oncohematological patients, with prior aim to reduce the incidence of early complications. In fact, we chose an echoguided approach to insert the catheter in a right supraclavicular site nearby the clavicular head of the sternocleidomastoid muscle. The 7.5-MHz probe was placed at the point of insertion of the sternocleidomastoid muscle onto the clavicle. The ultrasound technique allowed us a real time guide of the tip of the needle while it was inserted in the last portion of the internal jugular vein at the conjunction with the anoma vein. Thereafter all the patients underwent chest radiography. Up to now this route of insertion has been applied in 53 patients, in all the cases the catheter was successfully inserted. The only early complication was the appearance of a little hematoma at the site of insertion. No pneumothorax was registered. Twelve patients were affected by acute myeloid leukemia, 8 by acute lymphoid leukemia, 8 by acute myeloid leukemia, 8 by acute lymphoid leukemia, 1 by Hodgkin's disease, 15 by multiple myeloma, 4 by non Hodgkin's lymphoma and 12 by solid tumors. The catheter was inserted in 37 patients for chemotherapy, in 12 for PBSC collection, in 9 for autologous transplantation and in 2 for supportive therapy; some patients underwent more than one modality of treatment. Moreover, in 5 patients the CVC was inserted more than once during their clinical history. In all the patients at the beginning we inserted a 16-gauge catheter (Sekolan Seldy, Becketton Dickinson). When no peripheral veins were accessible for PBSC collection, the catheter was substituted, by Seldinger technique, with a 14 gauge-14 gauge bilumen Arrow catheter. We inserted the catheter with PLT<10,000/µL in 5 patients, with 10,000/µL PLT<20,000/µL in 4 patients; in both the settings no bleeding was registered. Moreover at the time of insertion 8 patients presented a neutrophil count <100/µL and three between 100 and 500/µL. In 5 patients the catheter was withdrawn because of appearance of complications: in one for an allergic reaction to the catheter, in four for infection. The catheters were substituted by the
We performed a cross-sectional study on quality of life (QOL) in patients with myeloid dysplastic syndrome (MDS) and bone marrow transplantation (BMT) in our Department. Because of the chronic nature and prognosis of MDS, QOL may be compromised thus its measurement could be useful for therapeutic planning. We have chosen the FACT-BMT Ver.4 questionnaire for cancer/hematological patients. After being granted permission by the author (D. Cellia, USA), we applied the modified Italian version on 34 consecutive patients with MDS, evaluating Hb levels and transfusion requirement as parameters of therapeutic effect. We performed descriptive statistics, parametric correlation and reliability analyses. Anemia varied from 40.8 to 87.7 years. Mean Hb was 9.5 g/dL (SD 2.1, range 6.0-15.0) and transfusion requirement was 1.0/month (1.4, 0.0-5.0). The QOL summary score was 90.7 (12.5, 66.0-114.0) with general part 67.8 (10.0, 50.0-88.0), specific part 22.9 (3.5, 13.0-28.0) and therapeutic outcome index (TOI) 53.1 (10.4, 33.0-74.0). When considering results obtained by FACT-BMT applied to American BMT-patients (M. Quelion et al., 1997), summary scores of total, general and specific parts showed better QOL in BMT-patients, especially before BMT, with respect to MDS-patients. When controlled for transfusion requirement, a significant correlation is found between Hb and requirement of total, general or specific summaries (p=0.03, p=0.98, p=0.04) but not with general part (p=0.49). The relationship with doctor gives highest reliability according to Cronbach’s a (0.88) whereas the lowest one (0.02) is for the specific part. In conclusion, QOL in MDS-patients is worse than to Cronbach's a (0.88) whereas the lowest one (0.02) is for the specific part. In conclusion, QOL in MDS-patients is worse than
and fruits. It is characterized by a high mortality rate because of rapid extension to contiguous sites and blood dissemination. We discuss the case of a patient with follicular non-Hodgkin’s lymphoma, presenting more than one-year severe neutropenia caused by therapies and disease, who developed a rapid and fatal fungal mucormycosis sepsis. A 42-year-old woman received a diagnosis of follicular NHL in April 1999 and received a fludarabine-based chemotherapy for six cycles with persistent pancytopenia since the fourth cycle. In August 2000, a combination of chlorambucil, vincristine and prednisone first and after the monoclonal antibody anti-CD20 (Mabthera) were administered. Two months after the end of immunotherapy, she developed cutaneous panniculitis like T-cell non-Hodgkin’s lymphoma. She rapidly progressed despite salvage therapy with CHOP regimen and ESHAP chemotherapy was administered. Subsequently, she developed a black eschar with induration, edema and erythema on the right arm and fever. Antibiotic therapy with meropenem, liposomal B-amphotericin and foscarnet was performed. After a few days she developed areflexia, paresis, slurred speech and lateral lingual swelling and, in few hours, positive bilateral Babinieres sign, coma and decerebration. Brain MRI showed an altered signal in T2 sequences on pontobulbar zone. Liquor was hemorrhagic with a granulocyte count of 60/mm3; culture examination was negative as was chemical examination. A punch biopsy of the skin lesion was performed and a Mucor spp. was identified. For the persistence of neurological signs, a brain MRI was performed again and showed an increase of the altered signal in T2 sequences without enhancement after gadolinium in the pontobulbar zone with mesencephalous and thalamus involvement, suggestive of an ischemic lesion, although the basilar artery seemed open. After three weeks of chemotherapy the patient died, in apparent clinical complete remission (complete rescue on WBC and ANC and regression of NHL skin lesions). Autopsy confined to the central nervous system, showed a Mucormycosis of the brain stem, dienephalus and pituitary gland with necrotizing meningoencephalitis, associated with a mycotic thrombus in the basilar artery. The fungi showed typical broad, not septated, haphazardly branched hyphae, which extensively penetrated into the walls of the blood vessels. The incidence of mucormycosis infection is approximately 1% in patients with hematological malignancy. First infection develops usually in upper and lower parts of the respiratory tract after inhalation of spores, to extend later; cerebral mucormycosis occurs as part of the systemic form, isolated (as in meningoencephalitis or space-occupying lesion) or as a rhinocerebral form; the cutaneous form is associated with a local sustained trauma. Diagnosis is performed by microbiopic examination. Isolated infection is usually successfully treated with a surgical debridement and treatment with amphotericin B. The systemic infection often has a fatal course. Neutropenia and amphotericin B therapy seem to correlate with recovery from infection. Mucormycosis should be considered in differential diagnosis, mainly in deep and prolonged neutropenia, in patients in the presence of infections with atypical features. Iconography will be shown.

The content of a clinical trial (CT) protocol nowadays follows standardized rules which facilitate the evaluation procedure and describes in detail the activities to be carried out during the test. The writing of a CT is therefore a complex activity in itself. It is accomplished through the collaboration of a team, which participates in the writing committee, generally working under the supervision of someone in charge of planning and co-ordinating the writing of the different sections of the CT. The physician responsible for the CT proposal has to control that the text is coherent and consistent in its parts also according to the rules of document presentation mentioned above. We consider the writing of a CT as a process in itself which can be described through a Workflow (WF), in which tasks and their interrelations are identified together with the different actors, who have to describe specific sections of the CT according to their competencies and responsibilities. The objective of our project is to define the functions of a system, which supports the entire life cycle of a CT. In this paper attention is focused on the definition process of a CT, on the description of the interaction between its sections using logical-semantic and temporal relationships, and on the integrated schema of a data dictionary. The life cycle of the CT can be divided into three processes: 1) clinical trial definition, which has as a result the design of a clinical trial with the entire protocol as a formal document; 2) clinical trial enactment, which means actually carrying out the trial; and 3) clinical trial evaluation, consisting in the final data processing and analysis, which leads to the final results. The process of enactment is managed by the WF management system, which supplies the planning and scheduling of the different activities, and controls their execution, taking into account the organization model and the available resources. Different health care structures participate in this process. They are geographically distributed, and they have to follow a uniform clinical behavior for the treatment of patients who are selected according to predefined inclusion criteria. The life cycle described above shows that clinical trials are analyzed from two different points of view: a) as a document, that is a textual description, which has to be evaluated and approved according to specific procedures; and b) as a schema, that is a graphical description of a WF, which models the process performed during the test in terms of activities as well as related logical-semantic and temporal links. For the graphical description we use a WF conceptual model, Atreus which has been successfully used in some heath care applications to model hospital processes as well as to describe guidelines for oncological patients. This model makes it possible to use a project methodology based on the re-use of the activities already described taking advantage of a WF library. In order to use already defined CT information managed by GiMEMA it is necessary to develop a data dictionary which provides an integrated view of the different types of data. The integrated schema has to be designed so that it enables: a) the re-use of the sections which form the textual description of the CT; b) the re-use of sub-sets of graphic schema of a CT; c) the modeling of the semantic relationships which connect the content of the sections through the Atreus model; and d) the definition of differentiat-
ed views of the CT, each one tailored on a specific type of user. This indicates that a system supporting textual and graphic descriptions is based on both DBMS and WFMS technologies. We are now developing a web page to navigate in the both textual and graphical descriptions, and a computer supported collaborative writing system dedicated to clinical trial protocols.

PO166
ACUTE PULMONARY EDEMA: AN UNUSUAL PRESENTATION OF PULMONARY ASPERGILLOSIS

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Case report. A 54-year-old woman with acute myeloid leukemia (FAB M1, secondary to myelodysplasia) received induction chemotherapy with fludarabine 30 mg/m² and cytosine-arabinoside 2mg/m² for five days (FLAG) after the introduction of a central venous catheter. She received antibacterial and antifungal prophylaxis with oral ciprofloxacin and itraconazole oral solution. Day +7: appearance of fever treated successfully with systemic empirical antibiotics (amicacin, ceftazidime and vancomycin). Standard X-ray was negative. The blood cultures were positive for Streptococcus mitis and Staphylococcus coagulase negative; both of them were sensitive to the antibiotics, and they became negative at day +13. Day +12: amphotericin B (1 mg/kg/day) was started because of the appearance of left thoracic effusion, several areas of parenchymal thickening in the interaxillary-hilar and supra-diaphragmatic regions of the right lung with a soft effusion at the right base. This treatment was interrupted after the third day because of severe intolerance. Aspergillus antigen was negative. Day +15: sudden appearance of acute pulmonary edema with palpitations, progressive dyspnea, rough cough, right parasternal pain and leg edema. Because of the suspicion of pulmonary thromboembolism we performed a lung scan with 99mTc-microspheres, which showed a perfusion defect in the posterior segment of the right lung, which suggested pulmonary thromboembolism. The echocolor-Doppler assay of the legs was negative for deep vein thrombosis. Therefore the patient was treated with systemic and oral anticoagulants, diuretics and oxygen-therapy with a complete clinical remission after 24 hours. Because of the persistence of rough cough and right parasternal pain in an afibrile state, we performed a high resolution CT scan which showed a parenchymal thickening of 3 cm in the right superior lung with cavitations, next to the costal pleura, linked to a segment of the right bronchial lobe, and another pleuro-parenchymal thickening in the ipsilateral costo-phrenic area. A broncho-alveolar lavage was performed because of suspected fungal infection; it was positive for some Streptococci viridantes only. So we performed a percutaneous pulmonary biopsy, which showed fungal hyphae compatible with Aspergillus. Therefore the anticoagulant therapy was stopped and antifungal therapy was started with itraconazole iv and then orally after discharge; during the following two chemotherapies we used, as secondary prophylaxis, Ambisome 3 mg/kg/day during aplasia and oral itraconazole 400mg at home. Clinical and radiological cure was obtained. Conclusions. This case report shows that the sudden appearance of acute pulmonary edema can be caused by embolic dissemination of Aspergillus.

PO167
“GLOVES AND SOCKS” PAPULAR PURPURIC SYNDROME FOLLOWING PRIMARY INFECTION WITH PARVOVIRUS B19: A LINK BETWEEN THE DERMATOLOGIST AND THE HEMATOLOGIST

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Gloves and socks papular purpuric syndrome is characterized by pruritic and painful edema and erythema localized to the hands and feet in a gloves and socks distribution, often associated with oromucosal lesions and fever. Several infectious agents, and especially viruses, have been implicated in the pathogenesis of this disease. We report the association between a primary parvovirus B19 infection and the occurrence of a new case of gloves and socks syndrome. The frequent occurrence of lymph node enlargement and leukopenia and thrombocytopenia make this entity of special interest for the hematologist. A 39 year-old man presented with the cutaneous lesions described above, but without mucosal involvement, in association with fever (38.5°C) lasting two days, and bilateral axillary and inguinal lymph node enlargement. The blood cell count revealed leukopenia (3300 leukocytes per cubic millimeter being the lowest value), with neutrophilia (neutrophils 71% and lymphocytes 12%) and thrombocytopenia (57000 platelets per cubic millimeter being the lowest value), while only a mild reduction of hemoglobin level (from 14 grams per deciliter to 12 grams per deciliter) was noted, with a reticulocyte count of 1%. Routine blood cultures for the most common bacterial, fungal and herpesviral agents, were negative. Serologic and molecular studies for viral infections, including all known hepatitis and herpes viruses, as well as Coxaschie A and B and echoviruses were invariably negative. The detection of IgM and IgG antibodies against parvovirus B19 was documented in serial serum samples collected during the acute and convalescent phases of the disease, clearly documenting seroconversion. The presence of B19 DNA sequences was detected in the serum samples from the acute phase, by polymerase chain reaction (PCR), confirming the association between B19 active infection (viremia) and the clinical symptoms. The cutaneous lesions and the lymphadenopathy disappeared after about 12 days, with a concomitant normalization of blood cell counts, without any therapy. Only 30 cases of gloves and socks syndrome have so far been described, 17 of which have been associated with a B19 infection. Our case reinforces the notion that B19 infection must be considered as the primary candidate in the differential diagnosis of possible causes of such a rare syndrome. Atypical aspects of primary B19 infection which need further investigations in this as well as in the reported cases of gloves and socks syndrome are the almost constant preservation of the erythroid lineage in the bone marrow, and the spontaneous remission and the lack of recurrence of the clinical manifestations, in absence of immunoglobulin therapy.
PO168
TRANSDERMAL FENTANYL IN THE MANAGEMENT OF CHEMOTHERAPY ASSOCIATED MUCOSITIS

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Fentanyl is a synthetic opioid with analgesic activity (morphine/fentanyl ratio 80:1) with transdermal administration. Four sizes are currently available with release rates of 25, 50, 75 and 100 mg/h. The TTS releases fentanyl at a constant rate for up to 72 h. After application of a patch, peak serum levels are approached within 17-24 h. Possible associated side effects are: respiratory depression, vomiting and nausea, constipation and CNS alterations. We report on our experience in the use of transdermal fentanyl in management of acute pain due to pneumo-nia in 1 patient with acute leukemia and to mucositis after induction treatment for acute leukemia in 9 patients. Patients suffered from mucositis requiring intravenous support (WHO-grade IV). Such a mucositis is usually controlled by administration of morphine. Sufficient analgesia was achieved with a dose of 50 mg/h in 4 patients, of 75 mg/h in 3 and 100 mg/h in 3 patients. Patients were treated for 9 days (median, range 3-20). Relevant side effects were not seen. Pain relief was evaluated by patients through a visual analog scale and by physicians through a 4-point score (poor, moderate, good, excellent).

<table>
<thead>
<tr>
<th>Pain Relief After Fentanyl (physician’s assessment)</th>
<th>2 patients</th>
<th>1 patient</th>
<th>5 patients</th>
<th>2 patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>Moderate</td>
<td>Good</td>
<td>Excellent</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions. Patients compliance and acceptance were excellent. Based on our experience with TTS fentanyl in this setting of patients, it seems to be effective and safe, easy to use, very well tolerated and to improve quality of life of leukemia patients.

PO169
THE ROLE OF THERAPEUTIC PLATELETPHERESIS IN THROMBOCYTOSIS

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Background. Therapeutic plateletpheresis (TP) is a procedure which often has a marginal role among supportive therapies of some hematological diseases. The high collection efficiency of the most up-to-date cell separators can make it a relatively simple, safe procedure. Patients and Methods. We administered TP to 3 asymptomatic outpatients, already treated with antiaggregating agents, for a total of 20 procedures: 1) Male, 71, lung carcinoma and thrombocytosis secondary to gemcitabine chemotherapy, platelet numbers (PN) 1,749×10^10/µL: 2 TP in 24 hours. 2) Male, 72, same disease as the first patient, PN 1,200×10^10/µL, multiple thrombotic precedents: 1 TP. 3) Female, 68 yrs, myeloproliferative syndrome, PN 3,393×10^10/µL, multiple thrombotic precedents and recent post-infarct splenectomy: 17 procedures in 100 days’ span of time, waiting for the efficacy of the therapies done (i.e.: first hydroxyurea, then busulphan, eventually optimal disease control by α interferon plus cytarabine). TPs were performed by Cobe Spectra LRS Turbo cell separator (Lakewood, CO, USA), adapting its software (version 7, LRS Turbo) to the therapeutic platelet (PLT) collection, raising, as high as possible, and up to the allowed maximum of 8×10^10/µL, the collection flow concentration, so as to reduce procedure time (PT), blood volume processed (BVP) and plasma volume harvested (PVH). The ACD/blood ratio was set to 1:6; 5% albumin in saline was used for plasma replacement. Results. Mean values regarding main parameters are listed in the table below:

<table>
<thead>
<tr>
<th>PT</th>
<th>PN (Pre)</th>
<th>PN (Post)</th>
<th>% of PLT reduction</th>
<th>PVH (mL)</th>
<th>BVP (% of total blood volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>94’ (±23)</td>
<td>1,710 (±576)</td>
<td>1,190 (±449)</td>
<td>31 (±6)</td>
<td>272 (±86)</td>
<td>Primary pattern: (877-3,393) (581-2,610) (18-42) (114-441)</td>
</tr>
<tr>
<td>55 (±3)</td>
<td>1,200 (±3)</td>
<td>1,200 (±3)</td>
<td>0</td>
<td>372 (±20)</td>
<td>Secondary pattern: 55 (±3) (37-120)</td>
</tr>
</tbody>
</table>

All TP were well tolerated by patients. Conclusions. When undertaking the decision to choose TP the evaluation of the measured PN should be joined to that of the patient’s clinical conditions and to an analysis of thrombotic (or hemorrhagic) risk. As a matter of fact, in asymptomatic patients, TP is often overlooked in favor of antiaggregating agents, whereas in symptomatic ones it is usually performed when PN is > 1,500×10^10/µL. However the rapid cytoreduction obtained by TP (even if waiting for the effect of any cytostatic joint therapies), can play a determinant role to prevent acute events in risk patients. Moreover, the low volumes of processed blood and removed plasma are not to be neglected, together with a more than considerable reduction of PN; this procedure can safely be given to elderly outpatients. These advantages are certainly the outcome of the high collection efficiency of the Cobe Spectra cell separator, which is equipped with innovative software.
Case Report

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Recent studies have demonstrated that low-molecular weight heparins (LMWH) are effective in the prophylaxis and treatment of deep-vein thrombosis (DVT). Skin necrosis is a rare but well-documented event during non-fractionated heparin therapy, observed usually after 2-5 days from the beginning of treatment.1 The mechanisms commonly implicated in the pathogenesis of skin necrosis are: platelet activation induced by heparin, type IV delayed hypersensitivity reaction, and presence of antibodies against heparin-platelet factor-4 (PF4) complex.2 Case Report. A 77-year-old female was admitted to our Division with a diagnosis of skin necrosis at the injection site of nadroparine. Platelet count was 81,000×10⁹/L, coagulation time, antithrombin III, protein S and protein C activity as well as activated protein C resistance were normal. Determination of antibodies against heparin-PF4 complex (ID-card Heparin/PF4 antibody test, DiaMed) was negative. The histology of skin biopsy showed a necrosis of papillary dermis, fibrinoid necrosis, and thrombosis of vessels. No signs of leukocytoclastic vasculitis were observed. Discussion. During the last decade, several reports have been related to unfractionated heparin administration with skin necrosis, usually at the subcutaneous injection sites. Recently, the appearance of skin necrosis during LMWH treatment has been reported. The present case describes the development of skin necrosis after subcutaneous LMWH injection with thrombocytopenia and without the presence of antibodies against heparin-PF4 complexes. In a review of the literature, skin necrosis after LMWH injection has been reported in about 5 cases. The main mechanism involved in the genesis of skin necrosis is the thrombocytopenia due to platelet aggregation related to the presence of antibodies against heparin-PF4 complexes. In conclusion, in our case despite the thrombocytopenia and the histologic demonstration of vessel thrombosis, we were unable to show the presence of specific antibodies to heparin-PF4 complex. These last data suggest a different pathogenetic mechanism of the platelet activation and aggregation responsible for the skin necrosis.

References
produce chronic synovitis and cartilaginous damage followed by irreparable arthropathy. Despite the conventional treatment (factor replacement and rehabilitation), synovectomy, surgical or medical, can reduce bleeding tendency and delay the development of full scale arthropathy. Synoviorthesis is the intra-articular injection of chemical or radioactive substances producing fibrosis of hypertrophied synovium. Methods. Between September 1999 and November 2000 we treated 20 severe hemophilia A and 2 severe hemophilia B out-patients with an average age of 34 years (15–60). Five patients (22%) presented FVIII inhibitor. The schedule includes intra-articular rifamicin once a week for five weeks (rifamicin 16 mg in 1 mL volume) without lidocaine or factor replacement. The indication for synoviorthesis was persistent synovitis and recurrent bleeding episodes. Results. A total of 29 joints were treated with 139 infiltrations. Twenty-four (83%) treatments were completed, 19 procedures were considered effective (79%), and insufficient in 5 (21%). Results were assessed by a test of self-evaluation for each patient. The best results were obtained in ankle treatment. Pain was reduced in 95% of cases, in 63% an improvement of the range of motion was referred and in 52% a decrease of concentrates was obtained. During the procedures 7 hemorrhages (5%) happened and in 3 of these we had to stop the treatment. Local pain was reported in 45%, but it was transient and did not need any type of analgesia. Conclusions. Chemical synoviorthesis with rifamicin appears to be effective for the treatment of chronic synovitis in out-patients. Major side effects were hemorrhathoses (5%) and pain in the site of infiltration. The procedure is at low risk for bleeding and flexible also for patients with inhibitors without any additional cost. We can not say if our infiltration program delays the arthropathy evolution for the short follow-up, but the any additional cost. We can not say if our infiltration program delays the arthropathy evolution for the short follow-up, but the delays the arthropathy evolution for the short follow-up, but the

Thus, AT-III might be able to directly affect adhesive properties of leukocytes. We have therefore studied the effects of AT-III on cell-cell adhesion of human neutrophils. Human leukocyte (0.5×10⁷/mL) aggregation was monitored as the increase in transmission of light through stirred suspensions in a platelet aggregometer. Aggregation curves were quantitated 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. Neutrophils (PM N) were purified 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 concentrate (Kybernin® Aventis Behring GmbH, Marburg, Germany). During the observation period (6 min) no aggregation of LPRP or PM N could be induced either with medium or with AT (0.2×10⁰ to 0.2×10⁻¹⁰ IU/mL). Additional presence of AT significantly inhibited PHA-induced aggregation of LPRP, whereas fMLP-induced aggregation was not affected by AT. In contrast, PHA-induced homotypic aggregation was augmented by AT, when no plasma and platelets were present. PM N fMLP-induced aggregation was not affected by AT. Though HSPGs are involved in the regulation of cells adhesion, in particular the homotypic cell-cell aggregation, AT was unable to affect in vitro aggregation of PM N after induction by the lectin, PHA, in the presence or absence of plasma and platelets. Obviously, specific interactions of AT with inflammatory cells require thorough work-up of the precise mechanisms involved.

PO174

EXTENDING THE "HOME TREATMENT PROGRAM" FOR ACUTE VENOUS THROMBOEMBOLISM TO HIGH RISK PATIENTS

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Home treatment (HT) for deep vein thrombosis (DVT) is becoming a widely accepted procedure in low-risk patients. We further tested the feasibility and safety of the HT program for acute venous thromboembolism (VTE) at our Emergency Room (ER), where 121 consecutive patients (84 with DVT and 37 with PE) were hospitalized for a few hours. According to the previously described algorithm (Siragusa et al., Haematologica 2000; 85:97), patients were screened as potentially eligible for HT or for standard in-hospital care. Low-risk patients (n=38) and those high-risk patients who refused hospitalization (n=15) were treated at home (enoxaparine 100 UI antifxa/kg/12h plus warfarin); the remaining high-risk patients (n=68) received the standard hospital care. Patients treated at home were followed at ER during the period of concomitant heparin and warfarin therapy. The results (table) indicate that there is no difference between hospitalized and HT patients in terms of major outcomes. This is even truer if one takes into account that a subgroup of high-risk patients was treated at home. After 3 months, 2 patients (standard in-hospital care) died of other causes than VTE. One patient (HT) developed a non-fatal intra-cranial hemorrhage. These preliminary results suggest that our HT program, based on a very short hospitalization, is as feasible and safe as standard in-hospital management.
Among the 18 patients with secondary CVT 1 had FV-L (2.5%). Among the controls 12 carried FV-L (2.3%) and 13 the FII-A gene, a recently identified hallmark of polycythemia vera. From peripheral blood was carried out in 18 patients; in 7 of them thrombocythemia in 2 cases). Thus among the patients exhaustively investigated the search for EECs improved the diagnostic yield for hematological causes up to 72.2% (13 of 18 patients).

### Table: Standard in-hospital vs. Home Treatment

<table>
<thead>
<tr>
<th>Event</th>
<th>Standard in-hospital</th>
<th>Home Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>40 DVT 28 PE</td>
<td>44 DVT 9 PE</td>
</tr>
<tr>
<td>Median age (range) in years</td>
<td>68 (37-92)</td>
<td>61 (22-90)</td>
</tr>
<tr>
<td>Proximal DVT</td>
<td>40 (100%) 14.2%</td>
<td>39 (88.6%) 5.5%</td>
</tr>
<tr>
<td>Distal isolated DVT</td>
<td>0 (0%) 7.1%</td>
<td>2 (4.5%) 0%</td>
</tr>
<tr>
<td>Thrombosis at the saphenous-femoral junction</td>
<td>0 (0%) 2 (7.1%)</td>
<td>3 (6.8%) 1 (11.1%)</td>
</tr>
<tr>
<td>Symptoms of PE</td>
<td>5 (12.5%) 3 (100%) 4 (9%) 9 (100%)</td>
<td></td>
</tr>
<tr>
<td>Active cancer</td>
<td>8 (20%) 5 (17.5%) 2 (4.5%) 1 (11.1%)</td>
<td></td>
</tr>
<tr>
<td>In-hospital stay</td>
<td>7 124 days</td>
<td>3.1 hours</td>
</tr>
</tbody>
</table>

### Events during the concomitant heparin and warfarin therapy

- **Duration of therapy (mean)**: 8.2 days 6.7 days
- **Recurrence DVT**: 0 (0%) 0 (0%) 0 (0%) 0 (0%)
- **Recurrence PE**: 0 (0%) 0 (0%) 1 (2.2%) 0 (0%)
- **Major bleeding**: 0 (0%) 0 (0%) 0 (0%) 0 (0%)
- **Minor bleeding**: 2 (5%) 0 (0%) 1 (2.2%) 0 (0%)
- **Heparin-induced thrombocytopenia**: 0 (0%) 0 (0%) 1 (2.2%) 0 (0%)

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**PO176**

**GAUCHER’S DISEASE TYPE 1, HEPATITIS C VIRUS INFECTION, CRYOGLOBULINEMIA AND LOW-GRADE NON HODGKIN’S LYMPHOMA**

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Gaucher’s disease type 1 (GD), the most prevalent lysosomal storage disorder, is due to β-glucocerebrosidase deficiency. It is characterized by the storage of uncleaved β-glucocerebrosides in cells of the reticuloendothelial system, leading to bone marrow infiltration, hepatosplenomegaly and skeletal lesions. Other clinical features include hematological changes with anemia and thrombocytopenia. This uncommon disease can be associated with various B-lymphocyte disorders, including malignant lymphoma, multiple myeloma, monoclonal gammopathy, chronic and acute lymphoid leukemia, and amyloidosis. The association of GD type I and non Hodgkin’s lymphoma (NHL) is extremely rare. In most reported cases the NHL was of B-cell type. We here report a case of GD type I associated with chronic hepatopathy HCV-related, cryoglobulinemia and NHL. A 34-year old woman came to our attention in February 2001. She had a history of Gaucher’s disease, for which a splenectomy had been performed in April 1991. The diagnosis of Gaucher’s disease type I was confirmed at the time by microscopic examination of the spleen and the bone marrow, and by a DNA study.

At the time of diagnosis, the laboratory investigation showed severe anemia (Hb 6.8 g/dL), and she received several blood transfusions. In November 1993, an enzyme-replacement therapy (ERT) with alglucerase (30 U/kg/month) induced a significant improvement of hematological parameters. In July 1997 the laboratory finding showed signs of liver damage: sGOT 220 U/L, sGPT 238 U/L, and a HCV infection (HCV-RNA >850,000 copy/mL) was documented in August 1997. In February 2001 the patient presented leukocytosis (WBC 17,500/mm³) with lymphocytosis (54%), sGOT 163 U/L, sGPT 161 U/L, and cryoglobulins (cryocrit 1.5%). Physical examination showed marked sub-mandibular, latero-cervical and bilateral supraclavicular lymphadenopathy, and hepatomegaly. CT scan and ultrasonography showed hepatomegaly (DL 16 cm) and two hilo-hepatic lymphadenopathies (20 mm). The patient refused a bone marrow and lymph node biopsy. However, an immunophenotype analysis of peripheral blood showed the presence of a monoclonal B-lymphoid population, CD5 negative. A therapy...
with Imiglucerase (60 U/kg/month) and α-interferon (3 MU/die) was started. HCV has been implicated as the etiologic factor for cryoglobulinemia, which may evolve, several years after diagnosis, to malignant lymphoma in about 10% of patients. In addition, an excessive chronic antigenic stimulation may be exerted on the immune system by the accumulating glucocerebrosidase, secondary to the distorted lipid metabolism. Therefore, it is possible that in this patient the development of NHL is secondary to a B-cell proliferation as a consequence of chronic antigen stimulation exerted by HCV infection, cryoglobulinemia and Gaucher’s disease. In this perspective, a combination therapy with Imiglucerase at high dosage, and α-interferon (antiproliferative and antiviral effects) could prevent lymphoproliferative disorders in patients with GD and HCV infection.

Aim.

To verify the usefulness of CyA therapy in refractory ITP.

Methods and Results

A study was carried out on long-term treatment (median 40 months) with CyA (3 mg/kg/d) in 12 selected adult patients with chronic, severe ITP resistant to all the usual therapies, splenectomy included. A median follow-up of 36.8 months (range 6-86) shows that CyA treatment obtained an improvement in 10 out of 12 patients (83.3%): 9 patients (75%) achieved a complete response (platelets in normal range) and one (8.3%) a partial response (platelet count of 80-120 × 10^9/L). Two patients (16.6%) were totally unresponsive. In 6 patients (50%) the discontinuation of CyA was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on the continued administration of the drug. The CyA intolerance was slight in spite of long-term treatment and no nephrotoxicity occurred. Other minor side-effects (slight creatinine level elevation, moderate hypertension, fatigue, paraesthesias, myalgia, gingival hyperplasia, hypertrichosis and tremor) were transient and always resolved spontaneously or with dose adjustment. Conclusions. Our study shows the safety and efficacy of CyA therapy in resistant ITP; this option seems reasonable in particular, dramatic, clinical situation, such as the unresponsiveness to all other treatments. In further selected cases, to avoid mutagenic effects or myelotoxicity, the CyA treatment could be attempted even before immunosuppressive chemotherapy.

Background. Physicians face therapeutic dilemmas when patients become resistant to known treatment in life-threatening conditions. A review of the literature shows a lack of comprehensive information on the clinical use of cyclosporin A (CyA) in the treatment of idiopathic thrombocytopenic purpura (ITP).

Methods and Results

A study was carried out on long-term treatment (median 40 months) with CyA (3 mg/kg/d) in 12 selected adult patients with chronic, severe ITP resistant to all the usual therapies, splenectomy included. A median follow-up of 36.8 months (range 6-86) shows that CyA treatment obtained an improvement in 10 out of 12 patients (83.3%): 9 patients (75%) achieved a complete response (platelets in normal range) and one (8.3%) a partial response (platelet count of 80-120 × 10^9/L). Two patients (16.6%) were totally unresponsive. In 6 patients (50%) the discontinuation of CyA was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on the continued administration of the drug. The CyA intolerance was slight in spite of long-term treatment and no nephrotoxicity occurred. Other minor side-effects (slight creatinine level elevation, moderate hypertension, fatigue, paraesthesias, myalgia, gingival hyperplasia, hypertrichosis and tremor) were transient and always resolved spontaneously or with dose adjustment. Conclusions. Our study shows the safety and efficacy of CyA therapy in resistant ITP; this option seems reasonable in particular, dramatic, clinical situation, such as the unresponsiveness to all other treatments. In further selected cases, to avoid mutagenic effects or myelotoxicity, the CyA treatment could be attempted even before immunosuppressive chemotherapy.
by macrothrombocytopenia and Döhle-like bodies in polymorphonuclear cytoplasm. MYH9 is the coding gene for non-muscle myosin IIa, which is the only myosin expressed in platelets and megakaryocytes. The pathogenesis of macrothrombocytopenia in subjects with MYH9 mutations is unknown. Myosin is a component of platelet cytoskeleton, which interacts with the COOH-terminus domain of some membrane glycoproteins (GPs). On this basis we hypothesized that MYH9 mutations could interfere with normal expression of platelet GP. Patients and Methods. Eight patients with MHA from 4 unrelated families have been studied. All of them had MYH9 mutations. GPs of platelet membrane have been investigated by flow cytometry with the following monoclonal antibodies (mAbs): SSEA (against GPIIIa), AP2 (against GPIb-IIIa), M845, E22 (against GPIba), PM25, S22 (against GPIb), SW16 (against GPIX), FA6-152 (against GPIX), and 49A (against GPIa). Binding of these mAbs has been revealed by a fluorescence-conjugated goat anti-mouse IgG. The values of mean platelet fluorescence of patients have been recorded and compared to those observed in a sample from a healthy donor that was run with the patients’ sample (the same healthy donor has been used for all patients). To obviate the confounding factor deriving from the larger volume of patients’ platelets, the ratios of fluorescence obtained in each patient with different antibodies have been studied along with the absolute values of fluorescence per platelet. Moreover in some experiments, subpopulations of platelets with similar volume have been identified in patients and control on the basis of forward scatter, and their fluorescence has been compared. Results. Comparison of absolute values of mean fluorescence per platelet in patients and donor was difficult because of the large differences in platelet volume. However, a 50-70% reduction of fluorescence with mAbs against GPIb-IX-V with respect to mAbs recognizing other surface molecules was observed in 6 of 8 patients. Analysis of platelet subpopulations selected on the basis of their volume showed that the relative reduction of GPIb-IX-V was greater in the platelets with the largest volume. Moreover, this analysis identified a GPIb-IX-V defect in the subpopulation of large platelets also in patients who were normal when whole platelets were studied. Conclusions. Surface expression of GPIb-IX-V complex is reduced in platelets from patients with MHA. The observation that a defect of GPIb-IX-V complex is present in MHA and Bernard-Soulier syndrome, both characterized by platelets larger than normal, suggests a possible relationship between GPIb-IX-V deficiency and macrothrombocytopenia. Since MHA is similar to heterozygous Bernard-Soulier syndrome (Blood 2001; 97:1330) in both clinical features and GPIb-IX-V deficiency, the differential diagnosis is not obvious.

PO180
THROMBOTIC THROMBOCYTOPENIC PURPURA: DOES THE APPLICATION OF THE PROTOCOL IMPROVE THE OUTCOME OF THE PATIENT?

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Introduction. Thrombotic thrombocytopenic purpura (PTT) is a rare syndrome characterized by consumptive thrombocytopenia, microangiopathic hemolytic anemia, fluctuating neurological abnormalities, fever and renal failure (40% of the cases). Pathogenesis is still unclear even if unusually large von Willebrand factor multimers play a crucial role in intravascular platelet aggregation. Improvement of therapeutic management, including a heightened awareness of the PTT syndrome and the identification of milder cases, allowed better survival rate (about 70-80%). Prognosis is conditioned by clinical presenting features (Rose score) and by the organizational level of the hospital. Survey. From January 1988 to March 2001, 19 pts (8 male and 11 females with mean age = 37 years range 18-66) were admitted to our hospital with a PTT diagnosis. Up to 1995 diagnosis has been structured in the Emergency Area with patient admission in specialized structures (hematology, hemostasis-thrombosis Center, or reanimation) with careful application of the GIPPT protocol. Results. Most meaningful data are reported in the tables below.

<table>
<thead>
<tr>
<th>Pts</th>
<th>Mean age</th>
<th>Diagnosis</th>
<th>Pts with score &gt; 4</th>
<th>% renal dysfunction</th>
<th>Mean N° of plasma-exchange</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988-94=7</td>
<td>33</td>
<td>42h</td>
<td>5/7</td>
<td>1/5</td>
<td>4</td>
</tr>
<tr>
<td>1995-01=12</td>
<td>44</td>
<td>47h</td>
<td>7/12</td>
<td>4/12</td>
<td>10</td>
</tr>
</tbody>
</table>

In the first group four deaths and two late relapses occurred. In the second group only one death, three early and four late relapses occurred. One refractory patient responded to splenectomy. Conclusions. The analysis of our study population has induced us to consider as crucial factors: the diagnostic delay, inadequate application of a plasma-exchange protocol. The careful adhesion to GIPPT protocol recommendations for treatment of TTP episodes permitted us to improve the outcome of our patients.

PO181
A SIMPLE AND SAFE NOMOGRAM FOR THE MANAGEMENT OF ANTICOAGULATION BEFORE SURGERY

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The management of anticoagulated patients before surgery is still controversial, surgeons fearing more hemorrhage than thrombosis, whereas hematologists the opposite. Although optimal treatment should be individualized balancing hemorrhagic and thrombotic risk, the introduction of a standardized protocol aimed to obtain a subtherapeutic INR for a short time before surgery could improve the practical management of such patients. We report here a simple nomogram for reducing warfarin dosage in order to obtain INR values between 1.5 and 2, which can be regarded as safe for many surgical procedures (C.
Kearon and J Hirsh, N Engl J Med 1997; 336:1506. We studied 80 consecutive patients (pts) (48 males, 32 females; mean age 62 years, range 24-92) on treatment with warfarin (INR ranging from 2 to 4) for atrial fibrillation (40), mechanical heart valves (29), deep-vein thrombosis dating back more than one month (11). The pts were scheduled for the following invasive procedures: oral surgery (16), ophthalmic surgery for cataract (16), prostatic biopsy (12), colonoscopy (11) or upper endoscopy (11) with biopsy, dermatologic surgery (10), laparoscopic hysterosalpingogram (4). In each patient we reduced preoperatively warfarin dosage according to the following protocol: 4, 3 and 2 days before procedure: half the usual dose; the day before: dose as usual; 6 hours after procedure: double dose. Starting from the day after, therapy was restarted as usual. INR was determined the same day of the procedure and one week thereafter; one month later a phone interview was performed in order to assess late complications. In 72/80 (91%) of pts preoperative INR values were within 1.5 and 2; 3 (3%) pts had INR higher (2.10 and 2.12) and 5 (6%) lower (1.45 and 1.42). No pts developed significant bleeding or thromboembolism either during the procedures, or in the postoperative period. At the one-week control 100% of pts returned to original INR values; neither hemorrhagic nor thromboembolic complications were observed at the one month phone interview. The small numbers of pts and the study design does not allow us to adequately assess the safety of protocol in terms of thromboembolic complications. Nevertheless we think that by avoiding the complete withdrawal of warfarin our protocol warrants partial protection against thromboembolism also during the brief time spent on subtherapeutic INR. Moreover, it avoids rebound hypercoagulable state after abrupt discontinuation of anticoagulant therapy. Therefore we conclude that this method for the management of anticoagulation before minor surgical procedures is safe and simple to use, avoiding the cumbersome shift of pts to either iv or sc heparin.

Results. All patients developed moderate Tp, with nadir on PODN. No coagulation abnormalities, such as consumption Tp were observed. Platelet-associated antibodies were always negative. RTC followed a predictable and reproducible pattern, with a steady increase by POD3. RTC increases heralded total platelet count increases by two days. Baseline CD62P and PAC1 expression was maximal around POD6. In the same samples platelets showed a markedly decreased response to ADP. Preactivated platelets were less prone to further in vitro stimulation, possibly due to surgery-related activation and consumption. TPO is markedly reduced in the control group (27.6±5.3 pg/mL, normal range 10-100 pg/mL). TPO regularly peaks on POD3, then gradually returns to baseline values by POD15. TPO peaked at the platelet nadir, just before percent and absolute RTC levels started to recover. Conclusions. Post-OLTX Tp correlates with liver functional insufficiency. Measurement of RTC by flow cytometry is a simple and accurate tool to study Tp due to consumption or reduced production. TPO measurement may be included in the functional assessment of liver function, especially if the patient has an indication to OLTX.

### PO182

**THROMBOCYTOPENIA AFTER ORTHOTOPIC LIVER TRANSPLANTATION. A PROSPECTIVE STUDY**

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Moderate to severe thrombocytopenia (Tp) is a frequent finding after orthotopic liver transplantation (OLTX). Tp lasts 5 to 7 post-operative days (POD), with a nadir around POD 4-5, and it usually recovers spontaneously within two weeks. Many studies have been performed to investigate possible pathogenetic mechanisms (i.e. increased destruction, immune-mediated or DIC-like mechanisms, dilutional loss, sepsis etc.), but results are equivocal. Ten OLTX patients have been prospectively studied with an extensive array of platelet and hemostasis testing, as listed here: 1. Clinical and post-transfusional events (thrombosis, hemorrhagic events, fever, infections); 2. Graft HD function testing; 3. Hemostasis testing, including d-dimer, fibrinogen, Antithrombin III and Platelet antibodies; 4. Complete blood cell counts; 5. Functional platelet studies by flow cytometry: a) Reticulated thrombocyte count (RTC) as percentage (normal range = 1-2%) and absolute count. b) Measurement of baseline platelet activation and functional reserve after ADP in vitro stimulation (baseline and stimulated quantitative expression of CD62P and PAC1 antigens); 6. Plasma thrombopoietin (TPO) levels by EUSA. End-stage liver failure patients served as control group (CTRL). Major laboratory findings are summarized in the table (mean values):

<table>
<thead>
<tr>
<th></th>
<th>CTRL</th>
<th>POD1</th>
<th>POD3</th>
<th>POD6</th>
<th>POD8</th>
<th>POD15</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLTs</td>
<td>70000</td>
<td>45000</td>
<td>17000</td>
<td>35000</td>
<td>72000</td>
<td>111000</td>
</tr>
<tr>
<td>RTCN</td>
<td>1.83</td>
<td>2.19</td>
<td>4.16</td>
<td>7.36</td>
<td>9.92</td>
<td>1.77</td>
</tr>
<tr>
<td>Baseline CD62P</td>
<td>3.08</td>
<td>2.54</td>
<td>4.33</td>
<td>15.85</td>
<td>11.42</td>
<td>3.35</td>
</tr>
<tr>
<td>Simulated CD62P</td>
<td>95.23</td>
<td>51.35</td>
<td>56.26</td>
<td>42</td>
<td>57.7</td>
<td>76.59</td>
</tr>
</tbody>
</table>

TPO pg/mL

|                | 34.8 | N.4 | 322 | 269 | 171 | 39 |

### PO183

**VINCRISTINE AS SALVAGE TREATMENT FOR RECURRENT THROMBOTIC THROMBOCYTOPENIC PURPURA**


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Thrombotic thrombocytopenic purpura (TTP) is a potentially fatal disease characterized by widespread platelet thrombi in the microcirculation, severe thrombocytopenia and variable neurological abnormalities. Treatment with plasma exchange (PE) or plasma infusion (PI) results in a survival of 70-90%. However, recurrence may occur, relapse rate ranging from 20 to 36%. Here we report treatment results from a series of 12 episodes of recurrent TTP which occurred in 7 patients, who received vincristine (VCR) as salvage treatment. Four relapses occurred in 1 patient, two in 2 patients and one in the remaining 4. The median age was 42 years (range 33-75) and all cases were classified as idiopathic. At relapse anemia and thrombocytopenia were found in all cases. In 7 episodes neurological signs were present. The median platelet count was 14±10^11/L (7-24), the median Hb value was 8.6 g/dL (6.8-10). Median serum LDH level was of 1305 IU/l (range 540-1870). The median time from discontinuation of previous treatment to relapse was 11 months (1-30).
2 patients VCR had been administered soon at diagnosis, given the absence of response to PE. VCR was given as follows: 2 mg on day 1 followed by 1 mg on day 4 and 7. After a 1-week interval, a second identical course was administered. In 10/12 episodes (83%), PI was concomitantly given at a dose of 15 µg/kg/day and discontinued when platelet count showed a stable increase in absence of clinical signs. In two episodes, occurring in a patient with severe cardiac failure, plasma was not given at all. Complete remission (CR) included normalization of hemoglobin, platelet, and serum LDH values as well as absence of clinical signs. CR was achieved in 12/12 (100%) episodes of TTP following VCR. In 8 cases CR was obtained after one week of therapy, while in the other 4 cases after the second week. No patient was refractory to treatment. The median CR duration was 15 months (2-16). There were 5 relapses in 3 patients; however, in all cases CR was achieved following retreatment with VCR. In 4 patients the disease did not recur at all. After a median follow-up of 15 months, all patients are alive and well. All patients were hospitalized during the first week of therapy, being treated as outpatients in the second week. The median number of days of PI was 7 (0-12). The median time to achieve a platelet count >150×10⁹/L and Hb>11 gr/dL was 7 (4-9) and 11 (4-15) days, respectively. The toxicity was acceptable: in 1 case (8%) leukopenia occurred (grade 2 according to WHO), while one patient, aged 74 years, experienced severe autonomic neuropathy leading to paralytic ileus, which resolved after 12 days. In 3 cases (25%) paresthesiae were observed. In conclusion, our data show that VCR is remarkably effective for recurrent episodes of TTP. The toxicity is acceptable and, compared with plasma exchange and other immunosuppressive approaches, VCR offers substantial advantage in terms of risk-benefit ratio. Finally, treatment is safe, inexpensive, and most patients may be treated on an outpatient basis.

**IDIOPATHIC THROMBOCYTOPENIC PURPURA AND SPLENECTOMY:**

**109 CASES FROM A SINGLE CENTER**

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Chronically idiopathic thrombocytopenic purpura (ITP) is characterized by thrombocytopenia due to platelet sequestration. The first line therapy with steroid induces complete (CR) or partial (PR) response in 70-80% of cases. However, the immunosuppressive therapy fails its efficacy in the majority of the patients after an initial response. In these patients, splenectomy is effective in about 70-80% of cases. In the present study we analyzed 109 patients with ITP, 36 males and 73 females with a median age at diagnosis of 34±16 years (range 6-67), who underwent laparoscopic splenectomy. One hundred and two patients underwent splenectomy after one or more therapeutic lines, while 7 patients were untreated. The mean number of platelets at the time of splenectomy was 36±31×10⁹/L with a mean interval diagnosis-splenectomy of 22±30 months. Overall 94/109 (86%) patients had a favorable response (CR+PR) after splenectomy. Fifteen (14%) patients were refractory and then underwent a second line therapy, which was effective in 10 cases. Twenty-two out 94 (23%) patients relapsed after 33±62 months (range 2-252) from splenectomy. Eighteen out 22 patients underwent further treatments which were effective in 12 cases. The mean follow-up after splenectomy was 176±81 (range 10-433) months. No infective complications were documented. Our experience confirms the efficacy and safety of splenectomy in chronic ITP, even though refractory or relapsed patients should be considered for a different timing of splenectomy or therapeutic approaches.

**RECOMBINANT ACTIVATED FACTOR VII CORRECTS THE BLEEDING RISK OF HOMOZYGOUS FACTOR XI DEFICIENT PATIENTS**


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FXI deficiency is a rare inherited coagulation disorder which is milder than hemophilia A or B, but with hemorrhagic complications after trauma or major surgical procedures. From therapeutic approaches no strict parallelism exists between FXI plasma levels and bleeding manifestations. It remains difficult to establish the level of FXI to aim for before surgery and to evaluate different prophylaxis programs especially in severe FXI patients. Basically, FXI deficient individuals receive fresh-frozen plasma (FFP) during the preoperative period with a possible risk of allergic reactions and blood-borne virus transmission. Even if a virally inactivated FXI concentrate is also available in few centers, this expensive product should be used with caution because of its possible thrombotic effect. A 38-yr old Sicilian female patient with homozygous FXI (<1%) deficiency, HBC and HCV positive, was recently referred to us prior to elective surgery. Her parents were first cousins. Four sisters of the propositus presented the same degree of FXI defect with variable bleeding tendency after trauma and/or surgery. The patient reported a first life-threatening bleeding episode during operation for ovarian endometrial cystoma despite generous FFP infusion together with antifibrinolytic agents nineteen years ago. In 1984 a second surgery of total hysterectomy for pelvic and uterine endometriosis was complicated by severe bleeding requiring multiple transfusions despite FFP units and tranexamic acid infusions. Successively, multiple tooth extractions were not complicated by any hemorrhagic event by using antifibrinolytics. In 1995 she underwent a cholecystectomy undertaken by laparoscopic procedure owing to abdominal adhesions, but despite FFP and antifibrinolytics administration a subphrenic vaste hematoma occurred. In April 2000 she was hospitalized for acute abdominal pain and icterus. The sonography of the abdomen documented gall-stones. Before the start of laparatomy, she was infused with human recombinant FVIIa (NovoSeven, Novo Nordisk A/S, Denmark) as a powerfull hemostatic drug. Tranexamic acid (5 g/daily) was administered until four days. The surgical procedure and the postoperative period were not complicated by any hemorrhagic event. In our opinion the human rFVIIa infusion in FXI deficient patients at highest risk of bleeding for surgery could enhance or sustain the formation of thrombin leading to a prolonged down-regulation of fibrinolysis by a more local hemostatic effect.
EVALUATION ON THE IMPLANT AND REMOVAL PROCEDURES OF THE PERMANENT/REMOVABLE ALN VENA CAVA FILTER. NEW PERSPECTIVE ON THE INDICATIONS FOR POSITIONING VENA CAVA FILTERS IN THE PROPHYLAXIS AND TREATMENT OF THROMBOEMBOLIC DISEASE

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F. Benedetti Valentini*
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The permanent/removable ALN caval filter belongs to the hydrodynamic family, is made of AISI 316 L stainless steel, has no welding points and is intended for either permanent or temporary use. The ALN filter is characterized by the absence of stasis volumes, by its very low occlusivity and by its capability to capture thrombi down to 2-millimeters diameter. Where the ALN filter is really innovative is in the possibility it offers to be used either as a permanent or as a temporary filter, as it can be explanted even after a long time. The 7-French introduction kit exists in the jugular, brachial and femoral approach versions. The ablation is carried out by means of a 9-French percutaneous catheter and can be performed only through a right jugular access. The filter is captured with a jaws system passed through the catheter which, when advanced, sheathes the filter. During the past 15 months a total of about 500 ALN filters have been placed in Italy, around 10% of which were removed. Our study reports the evaluation of 25 patients (males=16, females=9, median age 41 years, range 17-81) in whom an ALN filter was positioned; in 23 of them both positioning and removal procedures were performed; in the remaining 2 patients the ALN filter was repositioned in a supra-renal location after the original implant. The indications for positioning an ALN filter in these patients were: a) deep venous thrombosis and pulmonary embolism in 15 cases; b) prophylaxis in 4 pts with cancer who needed anticoagulant therapy; c) prophylaxis in 4 pts with cancer who developed thromboembolic complications to vena cava interruption in the thromboembolic disease and in surgical procedures associated with a high risk of thromboembolic complications.

LOCAL THROMBOLYTIC THERAPY IN CANCER PATIENTS AFFECTED IN URGENT NEED OF ANTIBLASTIC TREATMENT

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DVTs represent a relatively common feature in cancer patients because of release of thrombogenic proteins by malignant cells, compression on vascular vessels by tumor solid mass and sometimes because of insertion of a CVC for chemotherapy. The prompt diagnosis of DVT is crucial in order to solve this vascular complication and to cure cancer patients in time. In particular aggressive malignancies must be treated as soon as possible because the extension of the disease can be rapid and the prognosis is always poor especially if the chemotherapy is delayed. Another potential life-threatening complication in post CVC insertion DVT is septic embolism and the prompt lysis of thrombus as primary nucleus of infection could be crucial. In literature, there are actually no data that demonstrate LMWH or systemic thrombolysis is one of the most potential dangerous therapies we actually know because of the high risk of developing hemorrhagic and sometime fatal complications but local thrombolysis can be safe and feasible in specialized care units. We treated with local thrombolysis, two consecutive patients in urgent need of anticancer therapy affected by advanced stage malignancy who developed a DVT at the CVC insertion site. The diagnosis was clinical at first and confirmed by sonography and phlebography. The first patient, affected by IV stage mantle cell NHL developed a clinical DVT soon after the insertion of a CVC in the right subclavian vein. He was treated by Urokinase (UK) 100,000 U/h for 72 hours c.i. followed by LMWH prophylaxis (50 U/kg/12 hours) as maintenance treatment without any hemorrhagic complication: the lysis of the thrombus was complete after 72 hours thrombolytic infusion and the patient began the chemotherapy by the previous occluded CVC. The second one is affected by stage II NS variant Hodgkin’s disease who developed an antimicrobial large-spectrum resistant Staphylococcus epidermidis septic fever in subclavian DVT at the CVC insertion site. The patient was in the neutropenic phase after HDCT (BEAM) when DVT was diagnosed. The lysis of a large fragment of the thrombus by UK 100,000 U/h for 72 hours c.i. followed by LMWH 50 U/kg/12 hours prophylaxis permitted a rapid fever defervescence and the resolution of sepsis (hemocultures negative).

Referring to these preliminary reports, local thrombolysis can be recommended in selected oncologic patients. The procedure is safe in terms of risk of systemic hemorrhagic complications and discomfort for the patient. Lastly, this procedure, accelerating the adverse event’s cure, is cost effective.
PO188
PERIPHERAL BLOOD STEM CELL MOBILIZATION MAY INDUCE A HYPERCOAGULABLE STATE IN HEALTHY DONORS

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Blood stem cells (PBSC) mobilized with hematopoietic growth factors and collected from healthy HLA-matched donors have been increasingly used as the source of hematopoietic stem cells for allogeneic stem cell transplantation. However, some data have recently suggested that G-CSF may induce a transient hypercoagulable state. In addition, extracorporeal circulation is a well-known cause of hemostatic activation. Here, we prospectively investigated the effects of mobilization and collection procedures on the hemostatic system in 14 healthy donors given G-CSF. Material and Methods. Prothrombin time, activated partial thromboplastin time, fibrinogen, D-dimers, antithrombin III, protein C, protein S, thrombin-antithrombin complex (TAT), prothrombin fragment 1+2 (F1+2), coagulant factor VIII (FVIIIc), von Willebrand factor (vWF), thrombomodulin (TM), plasminogen (PLG) and homocysteine were evaluated in 14 healthy donors. Blood samples were collected from each donor before the G-CSF administration, immediately before and after leukapheresis procedures and 10 days after the G-CSF stimulation. Results. All donors showed normal values of above parameters before PBSC mobilization. Following the administration of G-CSF (10 µg/kg body weight for 5-7 days) and apheresis, we found an increase of TAT, F1+2, FVIIIc, vWF, homocysteine, and a decrease of AT III, protein C and protein S. The mobilization and collection of PBSC were well tolerated. Almost all stem cell donors reported some side-effects (bone pain, headache, chest pain and paraesthesia during the PBSC collection) but they were reversible within a short time. No donor discontinued the G-CSF application. So far no long-term effects have been observed in the follow-up. Conclusions. Allogeneic PBSC donors show evidence of thrombogen generation and a transient hypercoagulable state. Although the clinical implications of these findings remain unclear and larger studies are definitely required, donors at risk of thrombosis or hypercoagulable state should be followed carefully after G-CSF administration.
Partially supported by Regione Calabria and AIL.

PO189
RELATIONSHIP BETWEEN MENOPAUSAL STATUS, HORMONAL THERAPY AND tPA AND PAI-1 PLASMA LEVELS

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The fibrinolytic system consists of the circulating proenzyme plasminogen (PLG), which is converted to plasmin by the plasminogen activator (PA): tPA and urokinase; plasminogen-activator inhibitor type 1 (PAI-1) is a major inhibitor of the fibrinolytic system. Several studies have found an association between increased plasminogen activator inhibitor type 1 (PAI-1) and higher risk of atherosclerosis and its ischemic manifestations. In patients with deep venous thrombosis and pulmonary embolism elevated levels of PAI-1 have been reported, often in conjunction with low levels of endogenous tPA activity. Higher levels of PAI-1 were found in postmenopausal women than in premenopausal women. Although several investigations indicated that estrogen is responsible for the markedly decreased cardiovascular risk of postmenopausal women, the mechanism through which estrogen exerts its protective effect has not been adequately explained. In this study we investigated the effects of oral estrogen and progestin administration combined with transdermal estrogen therapy on plasma PAI-1 activity and tPA antigen levels. Two groups of healthy postmenopausal women were compared. The 31 women in the first group (mean±SD) 52.8±5.4 years) were randomly assigned to six months of the treatment; the 31 aged-matched women in the second group remained without hormone replacement therapy, and were employed as controls. Before and after three and six months of treatment, blood samples were collected from women of both groups. PAI-1 activity and tPA antigen levels were determined by a commercially available sandwich EUSA: Spectrolyse/tPA-1 Biopool AB and Tinelize tPA Biopool AB. In treated women mean (±SD) plasma levels of PAI-1 activity, significantly reduced from 14.9±6.7 UI/mL to 10±3.7 UI/mL after six months (p<0.05), no difference was found after three months (14.8±6.4 UI/mL). Plasma levels of tPA antigen increased significantly from 7.1±3.3 ng/mL to 7.9±3.6 ng/mL (p<0.05) after six months, while no difference was observed after three months (6.9±3.2). In postmenopausal women not receiving hormone replacement therapy, neither PAI-1 activity, nor tPA antigen levels changed significantly after three and six months. Our findings show that combined estrogen and progestin therapy was associated with increased fibrinolytic potential suggesting a cardioprotective effect of this therapy.

PO190
ASSESSMENT OF THROMBUS PROTEIN PRECURSOR IN PATIENTS UNDER ORAL ANTICOAGULANT THERAPY. OUR EXPERIENCE

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It is well-known that the monitoring of patients (pts) receiving oral anticoagulant therapy (OAT) is more useful to detect pts that are at risk of hemorrhage rather than pts at risk of developing thromboembolic complications, due to other mechanisms that cannot be modified by dicoumarol agents, and due to the absence of truly predictive tests capable of detecting a recent or forthcoming thrombosis event. The thrombus protein precursor (TTPTM) is a new test capable of measuring fibrin-soluble polymers (the immediate precursors of insoluble fibrin) that are indicative of an active-phase thrombosis. The TpPTM assessment in OAT pts aims at optimising the therapeutic intervention by correlating it with INR and with protein C, which as we know is inhibited by dicoumarol agents. We examined 59 pts (31 males and 28 females), with a median age of 63 (range 28-79), 23 of them had a mechanical valve prosthesis, 10 suffered from vari-
Reticulated platelets (RP) are defined as platelets with residual ribosomal RNA which is related to maturation stage. RP stained with thiazole orange (TO) are a useful marker for bone marrow thrombocytopoiesis and could be a simple test to distinguish thrombocytopoiesis between autoimmune and aplastic disorders. We evaluated RP of 22 patients (3 males and 19 females), median age 53 years (12-78), affected by idiopathic autoimmune purpura (TTP) at the moment of diagnosis and 61 healthy normal controls. RP were studied utilizing a quantitative fluorescent test on platelet rich plasma (PRP) obtained by centrifugation from peripheral blood. The platelets are fixed with paraformaldehyde, stained with TO and analyzed by flow cytometer. The results were examined by a fluorescence histogram where M1, M2, M3 regions were defined on the basis of the incidence of control platelet populations. The analysis of the results suggests that the number of RP was significantly higher in patients affected by TTP, compared with RP number observed in normal subjects (p<0.001). We obtained consistent TO positive platelet rates in patients with ITP when the platelet count was < 20 x 10^9/L compared with to patients with platelet counts > 20 x 10^9/L (p< 0.05). Furthermore, the number of RP resulted in a relationship with the platelet count: the presence of M2 and M3 fractions is inversely correlated with the platelet count (p=0.05). We concluded that our method of TO positive RP measuring is helpful for the diagnosis of ITP, especially when bone marrow aspiration is not possible and useful for the evaluation of bone marrow thrombopoiesis in different clinical applications.

PO92
TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA/HEMOLYTIC UREMATIC SYNDROME WITH PLASMA EXCHANGE. A REPORT OF 69 CASES

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Thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome (TTP/HUS) appear to be part of a spectrum of disease states that may share a similar pathogenesis. Formerly, TTP/HUS has been associated with 70-90% survival rates in large series of patients receiving plasma exchange therapy. Some authors have recommended the use of the cryosupernatant fraction of plasma to treat TTP/HUS refractory patients to fresh frozen plasma replacement. Other treatment modalities, such as steroids, vincristine, high-dose intravenous immunoglobulins, and splenectomy, have produced variable results. We examined 63 patients with TTP and 6 patients with adult HUS treated with plasma exchange in six institutions from 1987 to 2000. Complete remission was obtained in 50/69 patients (72.4%); 47 with TTP and 3 with HUS, after a median of 15 days (range 3-87). Partial remission and resistance to treatment were observed in seven patients (four TTP and three HUS, respectively). WHO were lost to follow-up. Death occurred in 12 patients (17.3%) after a median time of 11.5 days (range 3-40). Three patients died after complete remission for an underlying disease (two had cancer, one had SLE). Overall 15/50 (30%) surviving patients with TTP relapsed. Relapses occurred after a median time of 37.5 days (range 4 days to 9 years); it is noteworthy that 47.4% of patients relapsed within 30 days, 15.8% in the second month, 10.5% in the remainder of the first year and 26.3% in the subsequent years. Eight patients had more than one relapse. A striking finding in this study was the great variability in the treatment strategies adopted in the various centers. Such variability may be due to the lack of established treatment guidelines that can only derive from multicenter prospective randomized trials. The only therapy with proved efficacy is plasma exchange in this trial, but again it is not clear which is the best way to perform it, in terms of volume to exchange as well as frequency and duration of treatment. The 12 patients who received cryosupernatant as...
replacement fluid during plasma exchanges procedures did not have any obvious benefit. Six patients received platelet transfusions, despite the risk of worsening the disease, but their outcome was not different in comparison with the other patients. Another important issue is the increasing prevalence of relapses, probably because more patients recover from the initial acute episode due to improved supportive and specific treatments. Recent studies of vWF-cleaving protease inhibitor have improved our understanding of the disease pathogenesis and they could be of great value in identifying patients with high risk of relapse. These high risk patients undergoing clinical trials in future probably will determine the most effective and safe treatment.

PO193
HIGH PLASMA LEVELS OF FACTOR VIII:C AND XI:C ARE RISK FACTORS FOR VENOUS THROMBOEMBOLISM

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Established risk factors, including deficiencies of protein C, protein S or antithrombin, the factor V Leiden and prothrombin mutations, are present in about one third of unselected patients with thromboembolism. In addition to these inherited thrombophilic defects, elevated plasma levels of factors VIII:C and XI:C have been shown to be important in the pathogenesis of venous thromboembolism (VTE). Recently both antigen and one-stage clotting assay have been reported to be adequate methods for the evaluation of factor VIII as a prothrombotic risk factor. Aim of the study. To confirm that both antigen and clotting assays are adequate for the evaluation of high factor VIII:C plasma levels; to evaluate if factor XI:C one-stage clotting assay is adequate for evaluation of high factor XI plasma levels; to confirm that high plasma levels of factor VIII:C and XI:C are risk factors for VTE.

Methods. C-reactive protein (CRP), factor VIII:C and XI:C procoagulant activities (Instrumentation Laboratories) and factor VIII:C and XI:C antigen plasma levels (Cedarlane) were performed in 45 consecutive VTE patients and 45 sex and aged matched controls. All our assays were performed three months after the venous thromboembolic event. Correlation between procoagulant activities and antigen levels were calculated by using Spearman and Rank tests. Chi square statistical analysis was used to evaluate differences between the prevalence of high levels of factor VIII and XI in patients and controls. Results. CRP values were normal in all patients and controls: a good correlation was found between antigen and clotting assays for both factor VIII:C (r = 0.87) and XI:C (r = 0.80); Factor VIII:C plasma concentrations found in patients were arbitrarily stratified into three groups (<100%, 100-150%, >150%). For factor XI:C we used as cut-off the 95th percentile of plasma levels obtained in control subjects (122.5%). Fifteen out of 45 (33%) VTE patients and none out of 45 (0%) controls had factor VIII:C plasma levels > 150% (p = 0.0001). Among 45 patients, 20 (45%) had factor XI:C values that exceeded the 95th percentile, as compared with 2 (4%) out of 45 subjects in the control group (p = 0.0001). Conclusions. Factor VIII:C and XI:C procoagulant and antigen assays are both adequate methods for the evaluation of high plasma levels of factor VIII and XI as prothrombotic risk factors, but the procoagulant method is cheaper and quicker. Our data confirm that high plasma levels factor VIII:C and XI:C are risk factors for VTE.

PO194
TUMOR NECROSIS FACTOR α CANNOT BE USED FOR DIAGNOSING ACUTE VENOUS THROMBOEMBOLISM

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Venous thrombosis is known directly to elicit an inflammatory response in the thrombus and vein wall. The proinflammatory cytokine tumor necrosis factor (TNF) appears important in this vein wall response; such a response leads to amplification of thrombus formation. Upon this basis, it has been suggested that TNFα, as a marker of endothelial damage and thrombus amplification, might have a potential role in the diagnosis of acute venous thromboembolism (VTE) (Taheri et al., Angiology 1998; 49:537-41 and Angiology 1999; 50:703-6). Particularly, the authors demonstrated that urinary TNFα levels and its soluble receptor I are statistically increased in patients with confirmed deep vein thrombosis (DVT) and pulmonary embolism (PE). We investigated 104 patients referred to our Emergency Room for suspected DVT and/or PE; currently validated objective tests confirmed thrombotic events in 38 of them (36.5%, 95% CI 27.3-45.7). Five cc of plasma from each patient were collected at the time of diagnosis for determining TNFα levels (QTF1035 human TNFα Immunoassay Kit; Bioergonomics, St. Paul, MN, USA); the suggested average value was 2.20 pg/ml. The figure shows the diagnostic accuracy (by Receiver Operating Characteristic curve) of TNFα at different cut-offs (0.25, 0.5, 1.0, 2.20, 5.5, 10 and 25 pg/ml). Based on the ROC curve, the hypothesis that TNFα can be used as an effective tool for diagnosing acute VTE is not supported for any of the cut-offs considered.

PO195
CHANGES IN THE HEMOSTATIC SYSTEM DURING THE USE OF HORMONE REPLACEMENT THERAPY IN POST-MENOPAUSAL WOMEN


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Hormone replacement therapy (HRT) may reduce the risk of cardiovascular events in healthy post-menopausal women. However, recent studies suggest a 2-4 fold increased risk of idiopathic venous thromboembolism (VTE) among users of HRT. The aim of our study was to evaluate the overall effect of HRT on hemostatic variables probably related to increased VTE such as procoagulant-anticoagulant balance towards a procoagulant state. The changes in the hemostatic system could explain the increased risk of VTE in healthy post-menopausal women during HRT, nevertheless this risk could be higher in women known to have a congenital or acquired thrombophilic state.

The activation peptide was evaluated by the assays of prothrombin fragment 1+2 (F1+2) and plasmin-antiplasmin complexes (PAP) using ELISA techniques. Increased levels of FVIII:C and FVIII:C were observed in HRT users and HRT non-user women compared to controls (FVIII:C 126±58%, 120±59% vs 85±15% p=0.0001; FVIII:C 115±23%, 103±18% vs 90±16% p=0.0001). The activation peptides were significantly different compared to those found in control subjects; higher values were observed in HRT users compared to HRT non-users (F1+2=1.1±0.44 nM, 0.77±0.31 nM vs 0.45±0.35 p=0.0001; PAP=606±406 ng/mL, 514±205 ng/mL vs 235±59 p=0.0001). The ATTII and the PC were similar among the three different groups of subjects, but reduced levels of PS were observed in HRT users (PS 93±23%, 105±22% vs 109±12% p=0.0001). The mean normalized APC sensitivity ratio (APC-SR) was lower in the two populations of women as compared with that of controls (nAPC- SR 1.02±0.7, 1.02±0.8 vs 1.1±25 p=0.02). The values of free TFPI were reduced in HRT users compared to HRT non-users (9.1±1.9 ng/mL, 10.1±2.3 ng/mL vs 4.6±1.5 ng/mL p=0.0001). HRT appears to be associated with a shift in the procoagulant-anticoagulant balance towards a procoagulant state. The changes in the hemostatic system could explain the increased risk of VTE in healthy post-menopausal women during HRT, nevertheless this risk could be higher in women known to have a congenital or acquired thrombophilic state.

P0196
ACQUIRED COAGULATION DISORDERS AND THROMBOPHILIC PARAMETERS IN MULTIPLE MYELOMA PATIENTS

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Several reports indicate the presence of hemorrhagic as well as thrombotic complications in patients with MM. These complications have been generally attributed to the presence of the para-protein interfering with the normal mechanism of the coagulation system. The aim of this study was to investigate coagulation disorders and thrombophilic parameters for the detection of a thrombophilic state that has been associated, in a minority of patients, with a fatal outcome. To evaluate coagulation and thrombophilic state in patients with MM, the plasma samples of 61 consecutive patients with newly diagnosed M M were tested for the following parameters: PT, TT, fibrinogen, KCT, DRVVT, aPCR, PC and ATIII. There were 29 males and 32 females, median age 64 (range 33-72) yrs; 23 patients had MM and 38 patients had smoldering myeloma. Results were compared to those obtained in 62 normal matched controls (29 males and 33 females). Patients and controls were enrolled during a year period (from 04/1999 to 03/2000) at the Hematology section of our Department. In 23 MM patients, who had completed the antibiotic treatment, the values of the coagulation parameters present at diagnosis were compared to those observed at the end of treatment. A statistically significant difference for the number of prolonged PT (p=0.09) and PTT (p=0.02) was observed among MM patients and normal controls. Moreover, a reduced PC was observed only in 8 patients with MM and in no controls (p=0.02). Furthermore, 11 MM patients had a reduced ATIII as compared to only 1 observed in the normal controls (p=0.01). As for the values of the above parameters observed at diagnosis and after therapy, in the treated MM patients, all the 8 patients with prolonged PT, had a normalization of it (p=0.003).
Also 6/6 patients with reduced ATIII and 5/6 patients with reduced PC had a normalization of these parameters: p=N.S. and p=0.02, respectively. None of the 3 MM patients with an abnormally prolonged PTT had a normalization of this test. Our study suggest that in MM patients two principals pathogenic mechanism could play a role in coagulation disorders. One mechanism, responsible of the prolonged PT and PTT, may be linked to the interference of the paraprotein in the activation of the coagulation system. The other mechanism, responsible for the reduced ATIII and PC, may be linked to the latent nephrophathy present in these patients with a loss of proteins in the urine.

PO198
THROMBOPHILIC SCREENING IN YOUNG PATIENTS WITH ISCHEMIC STROKE

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Background. Ischemic stroke is a multicausal disease and its etiology remains unknown in approximately one third of patients despite extensive clinical and laboratory investigation. Patients and methods. Thirty-four patients (male = 14, female = 20; mean age 26.6 years, range 2-39) with documented ischemic stroke underwent prospective evaluation of antithrombin III, protein C, free and total protein S, activated protein C resistance, fibrinogen, factor VII:C and homocysteine levels. In all patients the presence of antiphospholipid antibodies was investigated by kaolin clotting time (KCT), diluted Russel’s viper venom time (DRVVT) and by research of anticardiolipin antibodies IgG and IgM (ACA-G and ACA-M). Prevalences of factor V Leiden, prothrombin variant G20210A and homozygosity for thermolabile variant C677T of the methylenetetrahydrofolate reductase (MTHFR) were evaluated and compared with those of 100 normal controls. Results. Antithrombin III and protein C were normal in all cases. One patient (2.9%) showed free protein S deficiency (49%) and three patients (8.8%) had activated protein C resistance. Homocysteine levels above 15 µmol/L were found in one patient (2.9%). Antiphospholipid antibodies were found in 23 patients (67.6%). Six (17.6%) patients and one (1%) normal control had combined inherited and acquired prothrombotic factors. Conclusions. The patient did not use donor blood transfusion whereas he did utilize autologous whole blood units in the following days. Conclusions. We think that, after this preliminary experience, for these difficult and complex clinical cases it is important to consider the opportunity to salvage blood intraoperatively and postoperatively and use autologous whole blood donation rather than autologous whole blood donation alone to reduce the need of donor blood transfusion.

PO199
BLOOD SALVAGE IN ASSOCIATION WITH WHOLE BLOOD AUTOLOGOUS DONATION FOR TWO HIGH RISK SURGERY PROCEDURES

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In last years, transfusional risks have stimulated more interest for new solutions to avoid or to limit the need of donor blood. Among many solutions, the possibility to save and than to reinforce autologous blood is most interesting. For this reason the salvage of autologous blood intraoperatively and postoperatively, especially for major surgery, is spreading very quickly. Now we report two clinical cases of hip and knee replacement with high transfusional risk, where the utilization of salvage of autologous blood intraoperatively and postoperatively (RS), in association with autologous whole blood program donation (PSA), avoided donor blood transfusion. The salvage, intraoperatively and postoperatively, was possible with automatic cell separator in continuous flow FRESENIUS C.A.T.S. that simultaneously draws, washes and produces pure red blood cells saved from the operative field. Clinical case #1. Patient (70 years old), planned for hip replacement, sent to us from another Hospital because not suitable for autologous whole blood donation because of ischemic cardiopathy and showed type and screen positive for an anti celliano antibody.

PO200
CATASTROPHIC VASCULAR OCCLUSION SYNDROME IN A PATIENT WITH GAUCHER’S DISEASE TYPE I AND ANTIPHOSPHOLIPID ANTIBODIES

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Gaucher’s disease (GD) type I (β-glucocerebrosidase deficiency) is characterized by the storage of uncleaved β-glucocere-
brosis in the cells of the reticuloendothelial system leading to bone marrow infiltration, hepatosplenomegaly and skeletal lesions. Hematological changes with anemia, thrombocytopenia and thrombocytopathy are common. Recently clotting factor and natural inhibitors deficiencies have also been reported as well as increased levels of antiphospholipid antibodies (APA) but the pathophysiology of such abnormalities is still unclear. We report the case of a 48-year old man who developed massive venous thrombosis 5 years after receiving a diagnosis of GD type I with concurrent APA, lupus anticoagulant (LAC) and anticardiolipin antibodies (ACA). At admission the patient, splenectomized and in enzyme replacement therapy (ERT) at the low dosage-high frequency regimen (Imiglucerase 15 U/kg/month), complained of rest dyspnea, abdominal pain, lower limbs edema and abdominal distension. A computed tomography of the chest and abdomen showed total thrombosis of the jugular common vein, brachiocephalic left vein, intra-extra hepatic portal vein and of the proximal tract of the mesenteric vein, massive hepatic atrophy, ascites and pleural effusion. Levels of protein S, protein C and antithrombin III were normal, mutation for the allele of methylene tetrahydrofolate reductase (MTHFR-C677T), prothrombin 20210 and factor V Leiden were not found. Prothrombin time was slightly prolonged; D-dimer and fibrinogen were increased. Activated partial thromboplastin time (APTT) was prolonged (55°-n.v.=30°) and not corrected by mixing procedure (Ratio=1.7). Diluted Russell’s viper time (dRVVT) was prolonged and ACA were significantly increased (4,7U/mL n.v. <1,5). APA are a heterogeneous group of antibodies that are detected in the serum of patients with a variety of conditions, including autoimmune diseases (LES), infections (AIDS), lymphoproliferative disorders and recently have also been reported in GD. Thromboembolic events, thrombocytopenia and recurrent fetal loss are the most frequent clinical manifestations. GD is associated with a significant increase in specific autoantibodies, which may be the result of polyclonal stimulation secondary to the distorted lipid metabolism. In summary we think that global immune dysregulation, which is found in GD can promote APA formation and that in these patients ERT at high dosage may correct the immune dysregulation and may prevent the development of autoantibodies.

PO201
ASSSESSMENT OF THE ASSOCIATION BETWEEN THROMBOTIC RISK FACTORS AND CLINICAL EVENT
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We saw 145 patients (pts) in our department (53 males and 92 females with a median age of 44); 70 pts had arterial and/or venous thrombosis while the remaining 75 pts appeared to be healthy and homogeneous with respect to their demographic traits. Of the thrombosis cases, 57% involved the central nervous system and the retina, 41% involved peripheral vessels, while 24% of the cases were ischemic heart disease and other minor conditions. A case-control study was carried out in order to assess the significance of the association of some hereditary thrombophilic factors (i.e. factor V Leiden, factor II variant) with the thrombotic condition. The association of the concomitant presence of acquired risk factors (birth control pill, diabetes, hypertension), hereditary risk factors and the thrombotic condition was also assessed. The results obtained were as follows:

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The odds ratio (OR) for the genetic risk factor alone is not significantly deviated from unity. This fact seems to confirm the multifatorial origin of the thrombotic event and of the acquired risk factor importance. The association existing between the condition and the concomitant presence of genetic factors (VL or II variant), and the clinical condition (platelet hyperaggregation and/or presence of anti-phospholipid antibodies) was also significant (OR: 1.8 and 4.0, respectively). We examined collaterals, descendants and ascendants of 10 subjects with twofold congenital defect, multiple- and multidistrict thromboses and we observed an FVL positivity in 7 probands out of 24, an FII variant positivity in 11 probands, while 3 probands displayed both positivities. The association between APC-R (present in 16% of the pts) and FVL (present in 86%) was confirmed.

PO202
RECURRENT THROMBOEMBOLIC EVENTS AND PROTEIN C DEFICIENCY IN A PATIENT WITH UNSTABLE Hb GENOVA
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Unstable hemoglobins are structural variants inherited as dominant autosomal disease. Affected subjects suffer from chronic hemolytic anemia with variable clinical expression linked to a point mutation inside the hemoglobin molecule. In abnormal Hb Genova (β28(β10 Leu →Pro) the proline substitution in β10 is placed towards the middle of the helix and the presence of an iminoacid would disrupt a helical sequence causing chain instability associated with inclusion bodies of the red cells and severe hemolytic anemia. The variant was described for the first time in this patient and her mother in 1967.1 Subsequently 13 subjects in 6 families of different ethnic ancestry having the same defect, the majority due to spontaneous mutation, have been reported. All carriers suffered from hemolytic crises after infectious episodes with variable improvement after splenectomy. Our patient, closely followed-up since infancy, has benefited from this procedure regarding the hemoglobin value, nevertheless frequent deep venous thrombosis of the legs and pulmonary embolism have complicated the clinical course. Coagulation and genetics tests performed to evaluate hypercoagulable state shows severe protein C defect. Screenings for FV Leiden mutation, prothrombin G20210A mutation and homocystein level are normal. Because in reported cases of patients with Hb Genova thromboembolic events were not found, we suggest that thromboembolism in our patient is linked to the severe protein C defect The patients is treated with life-long warfarin therapy.

PO203
ANTIPHOSPHOLIPID SYNDROME: CASE REPORT

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A 72-year-old woman with COPD and atrial fibrillation, who had been taking warfarin because of repeated transient ischemic attacks, was admitted to our hospital for severe acute cardiac and respiratory failure with hypoxemia and hypocapnia, hepatocellular dysfunction, acute renal failure, elevated LDH levels, acquired coagulopathy with prolonged PT and PTT, minimally increased D-Dimer levels, severe anemia and moderate thrombocytopenia, acute chest and epigastric pain. Myocardial infarction was excluded and a chest film showed diffuse alveolar infiltrates compatible with pulmonary edema and bilateral pleural effusions. Echocardiography revealed right ventricular overload with pulmonary hypertension and no alterations in ventricular wall motion pattern. ARDS was considered and warfarin was continued. A lung perfusion scan did not reveal gross pulmonary embolism, even if the examination was not reliable on the whole; anyway heparin was given intravenously at a daily dose of 25,000 units. Hematologic evaluation considered a microangiopathic hemolytic process, confirmed by a negative Coombs’ test, reticulocytosis, schistocytosis on the blood smears and low haptoglobin levels. A hypercoagulability syndrome was high on the diagnostic list and blood was obtained for anticardiolipin antibody sampling for confirmation. The patient continued to have dyspnea and bouts of abdominal pain with distension; so she underwent pulmonary and mesenteric angioMRI, which revealed no macrovascular changes. A thrombotic microangiopathy and the highly suspected antiphospholipid syndrome were then confirmed by a positive test for anticardiolipin IgG antibody. The patient’s clinical course was complicated by recurrent mesenteric and peripheral arterial ischemia; so, despite heparin therapy and cardiorespiratory support, she died after developing seizures and coma.

PO204
INCREASED MENSTRUAL BLOOD LOSS IN WOMEN TREATED WITH ORAL ANTICOAGULANTS

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In women with inherited bleeding disorders (as in von Willebrand disease) menorrhagia is quite common. Oral anticoagulant therapy (OAT) interferes with the synthesis of the vitamin K-dependent coagulation factors (F II, F VII, F X, F IX); it is not clear whether OAT might increase menstrual blood loss in women treated with these drugs. A questionnaire was given to 46 patients in fertile age who received OAT for at least six months, with the purpose to verify if menstrual blood loss was increased on OAT. Twenty-four of 46 patients has an increase of menstrual blood loss; however only 8 patients presented a true menorrhagia with significant decrease of Hb and Hct. OAT seems to increase menstrual blood loss in menstruating women; larger studies are necessary to evaluate the impact of this feature on the quality of life and compliance to therapy of women on OAT.

PO205
A CASE OF SPLENIC INFARCT COMPLICATING NON-RHEUMATIC PAROXYSMAL ATRIAL FIBRILLATION

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Splenectomy is a rare complication of atrial fibrillation (AF), a common arrhythmia, particularly in the older age groups, that confers an increased risk of systemic thromboembolism to these patients. We describe the case of a 72-year-old man who presented to the Emergency Department with a four-day history of intermittent left upper abdominal pain. Since 1995 he had been treated with calcium antagonists and amiodarone for hypertensive cardiovascular disease and paroxysmal non-rheumatic AF. On examination he was acutely ill with an enlarged tender spleen. EKG showed AF; plain abdominal X-rays showed moderate distension of a bowel loop in the left abdomen. WBC were 14,700/mL with 80% of neutrophils. The patient was admitted to the Surgery Department with a diagnosis of acute diverticulitis and he was treated with i.v. fluids and analgesics. The pain was unmitigated and the day after an abdominal ultrasonography disclosed an enlarged spleen (14 cm) with a hypoechic region consistent with splenic infarct. A CT scan of the abdomen showed an hypodense area of infarction 10×9.6×5.6 cm in diameter with liquefactive necrosis in the spleen and multiple smaller ischemic areas in the right kidney and in the liver. A moderately enlarged left atrium was seen on echocardiogram. Serum LDH was 1348 U/L. Treatment was started with enoxaparine, i.v. antibiotics and propafenone, oral anticoagulants. Sinus rhythm was promptly restored and the patient was discharged after six days, when a subsequent CT scan showed a reduction of the massive splenic infarction. Comment. This case of splenic infarction complicating paroxysmal AF was reported by the authors mainly to stress that: 1) conservative medical treatment is possible and successful in similar cases; 2) the clinical picture of splenic infarction is non-specific and can simulate more frequent causes of acute abdomen seen in clinical practice (acute diverticulitis in our patient); 3) clinicians should become highly suspicious of splenic infarction in a patient with AF and upper left abdomen pain, when laboratory tests reveal leukocytosis and marked increase in serum LDH concentrations. In fact, we have found raised levels of the enzyme in previous similar reported cases; 4) ultrasound (US) is a very useful means of noninvasively examining the spleen and can represent an appropriate first-line investigation for suspected splenic infarction; 5) US and CT scan of the abdomen can demonstrate multiple clinically silent ischemic lesions, mainly in the kidneys and in the liver, in patients with systemic thromboembolism due to atrial fibrillation.

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FAMILIAL THROMBOPHILIA AND OBSTETRIC COMPLICATIONS

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Background. The association of thrombophilia with obstetric complications is well known. Pregnancy is a hypercoagulability state. In women with thrombophilic mutations, insufficient placental perfusion, due to fibrin deposition, is a major underlying pathogenetic mechanism to explain the obstetric complications. M. Matera and M. Methods. From July 2000, thirty-two women (mean age 30 years, range 25-38) with a previous history of obstetric complications (preeclampsia n=8, recurrent pregnancy loss n=20 and missed labor n=4) were investigated for the presence of factor V (FV) Leiden, prothrombin G20210A mutation, MTHFR TT 677 genotype. Moreover, IgG and IgM anticardiolipin antibodies, IgG and IgM anti-β2 GP1 antibodies, antithrombin, protein C and protein S were determined in all patients. Women were tested 60 days after the delivery or after the pregnancy loss. Results. The following table shows the incidence of genetic disorders evidenced in this set of patients.

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In one out of the four cases of missed labor, there was coexistence of two mutations (FV Leiden heterozygote, MTHFR TT 677 genotype homozygote). In three women with recurrent pregnancy loss, anticardiolipin and anti-β2 GP1 antibodies were elevated. In all patients protein C, protein S and antithrombin were normal. There was no association between congenital and acquired risk factors. Conclusions. Our results show an association between prothrombotic genetic factors and obstetric complications in a selected group of patients. The diagnosis of these risk factors might be the tool to decide correct prophylaxis (aspirin or low molecular weight heparin) associated with sequential ultrasonographic Doppler and frequent obstetric follow-ups. Many authors suggest folic acid administration six months before attempting conception in women with MTHFR C677T mutations.

evaluation of chimerism in non myeloablative bone marrow transplantation by STR/VNTR polymerase chain reaction

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After bone marrow transplantation the incomplete engraftment and the persistence of recipient hematopoietic cells create a coexistence of donor and host-type hematopoiesis which is named mixed-chimerism. This phenomenon is particularly important in the patient transplanted for hematological diseases such as leukemia, myeloma, lymphoma, because the presence of recipient cells might disclose reappearance of the malignant clone. Recently experimental and clinical experiences have demonstrated successful donor engraftment following non-myeloablative preparative regimens. These less toxic conditioning strategies may be better tolerated with diminished transplant-related morbidity and mortality. In order to evaluate the possible dynamics of chimerism after non myeloablative BMT we established a quantitative STR (short tandem repeats) or VNTR (variable non tandem region)-PCR approach. Standardized mixed chimerism samples were generated by mixing pre-transplant recipient and donor DNA in a range of percentages for each individual case. PCR analysis for the informative locus was carried out on sequential patient samples using the informative primers. After separation on the polyacrylamide gels and silver staining, for the STR-PCR, or electrophoresis on the agarose gel and staining with ethidium bromide, for the VNTR-PCR, signals were analyzed by Gel Doc Analyzer and results were taken on the basis of individual standard curves. Post-BMT DNA samples were investigated in sequences and the signal intensities were compared to the standard curves. We analyzed post-BMT DNA from total bone marrow and from isolated populations such as granulocytes, lymphocytes CD3+, monocytes CD14+ obtained by FACS sorting. We investigated 13 patients treated with non-myeloablative bone marrow transplantation from the Transplant Centers of Turin and Alessandria. The first analysis was performed at day 30, the subsequent at day 60, at day 90 and day 180 after BMT and so on. In all cases we performed the standard curve for almost two informative microsatellites. At day 30 8 out of 13 samples presented 20-30% of recipient cells in CD3+ subpopulation, the remaining 5 samples presented less than 10% of recipient cells in CD3+ subpopulation. One case which presented at day 30 30% of recipient cells in CD3+ subpopulation, at day 60 presented 50% of recipient cells both in CD3+ subpopulation and in total bone marrow while displaying only 30% of recipient cells in the granulocytes population. At day 90 all the populations investigated, total bone marrow, peripheral blood, granulocyte and CD3+ subpopulations presented less than 50% of donor cells suggesting a relapse of disease. In the other cases a condition of full donor chimerism was achieved; in these cases more than 97% of donor cells were detected. In conclusion, even if our data are preliminary, we could...
assume that the STR- or VNTR PCR is a simple approach which allows us to investigate the presence of recipient cells in different hematopoietic subpopulations and in this way we are able to identify a possible relapse of disease also in those patients in whom it was not possible to detect a specific molecular marker.

PO208
IMMUNOPHENOTYPIC PROFILE OF AC133-POSITIVE CELLS IN BONE MARROW, MOBILIZED PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD

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In the hematopoietic system, AC133 expression is restricted to a subset of CD34+ progenitor/stem cells with long-term repopulating ability. On this basis, AC133 has recently emerged as a target of an alternative way to identify and separate early hematopoietic progenitor cells. We analyzed, by means of flow cytometry (FACSCalibur, Becton Dickinson, USA), 15 umbilical cord blood (UCB) samples, 15 bone marrow (BM) samples from healthy donor (#5) and from subjects suffering from non-Hodgkin’s lymphoma without marrow involvement (#10) and 15 peripheral blood (PB) samples from cancer patients undergoing mobilizing high-dose chemotherapy followed by daily subcutaneous administration of G-CSF (5 µg/kg body weight), aiming to assess the immunophenotypical profile of AC133+ cells. The mean percentage number of AC133+ cells in mobilized PB, BM and UCB was 0.56±0.26% (range 0.2-4.4), 0.54±0.14% (0.3-0.7) and 0.18±0.11% (0.06-0.4), respectively. In addition, in mobilized PB we observed a greater number of AC133+ cells co-expressing CD34 (p<0.0001), HLA-DR (p 0.001) and CD34 (myeloid commitment) (p<0.0001) as well. However, in BM a greater number of AC133+ CDw90(Thy-1)+ cells was found (p 0.019). Finally, committed T (CD7+) and B (CD19+) AC133+ cells were detected principally in UCB (p<0.0001). In conclusion, AC133+ cells show a heterogeneous immunophenotypical profile. The existence of different AC133+ subpopulations might be relevant both to ascertain and predict the rapidity and long-term durability of recovery after myelosuppression and rescue with hematopoietic progenitors.

PO209
BONE AND BONE MARROW INTERACTIONS: HEMATOLOGICAL ACTIVITY OF OSTEOBLASTIC GROWTH PEPTIDE-DERIVED CARBOXY-TERMINAL PENTAPEPTIDE I: MOBILIZING PROPERTIES ON WHITE BLOOD CELLS AND PERIPHERAL BLOOD STEM CELLS IN MICE

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Osteogenic growth peptide (OGP) increases blood and bone marrow cellularity in mice, and enhances engraftment of bone marrow transplant. Carboxy-terminal pentapeptide (OGP10-14) has several properties of full-length polypeptide. We evaluated if synthetic OGP-derived pentapeptide (sOGP10-14) has some activity on peripheral blood cell recovery after cyclophosphamide-induced aplasia, and on stem cell mobilization. Peripheral blood stem cell (PBSC) mobilization was evaluated by administering granulocyte colony-stimulating factor (G-CSF) or sOGP10-14 after cyclophosphamide (CTX) injection. Hematological parameters and CD34/Sca-1 positive cells were sequentially evaluated. Colony-forming tests were performed in bone marrow cells from CTX-, G-CSF- and sOGP10-14-treated mice. sOGP10-14 was able to enhance band cells and monocyte recovery after cyclophosphamide administration. White blood cell (WBC) counts reached the maximum peak by day +10 but, on day +7, a significant recovery was already detected in sOGP10-14 treated mice. On day +10 the WBC increase in sOGP10-14-treated mice was comparable to that found in G-CSF treated ones. Moreover, CD34/Sca-1 positive early precursors were significantly mobilized by sOGP10-14 compared to the control group. In sOGP10-14-treated mice, the colony-forming unit-granulocyte-macrophage-megakaryocyte (GEMM-M-CFU) and burst-forming unit-erythroid (BFU-E) was significantly increased in bone marrow cells in comparison to mice treated with CTX only.

PO210
SINGLE-CENTER EXPERIENCE OF DETECTION OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH AML/ETO-ASSOCIATED ACUTE MYELOID LEUKEMIA BY REAL-TIME RT-PCR

Institute of Hematology and Medical Oncology Seràgnoli, University of Bologna

The t(8;21)(q22;q22) translocation is found in approximately 10-15% of cases of acute myeloid leukemia (AML), and is frequently the only cytogenetic abnormality present in the leukemic blast. The t(8;21) has been reported to be associated with a relatively good prognosis. Qualitative RT-PCR can be used for the detection of residual t(8;21) positive cells in patients. However, several studies using qualitative RT-PCR for the detection of minimal residual disease have produced contradictory results. Quantitation of residual AML1-ETO fusion transcript during complete remission (CR) may therefore provide a better indication of relapse or possible cure. In contrast to end-point quantitative methods, the fluorometrically-based real-time RT-PCR technique (RQ-PCR) allows simple and rapid quantitation of the target sequence during the extension phase of PCR amplification. We report seven patients with t(8;21)(q22;q22), who as well as being routinely addressed to cytogenetic and qualitative RT-PCR monitoring in the Seràgnoli Institute at diagnosis and during the follow up, were also retrospectively studied by RQ-PCR. All seven patients showed around 1000 copies of AML1-ETO/ABL ×106 cells ABL (using ABL as the control gene) at diagnosis. Four patients showed a 2-log decrease following successful induction chemotherapy. Three patients showed a 1-log decrease after induction. Two out three patients showed a further 1-log decrease after consolidation. The remaining patient later relapsed following an increase in the copy number. Our experience provides an example of how RQ-PCR may be used to assess early response to treatment and, perhaps, by pattern analysis, to predict ultimate clinical outcome.
**30 Ore per la Vita** A.I.L. grants.

**PO211**

**MOLECULAR EVALUATION OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA**

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Few studies report about quantitative molecular evaluation of the minimal residual disease (MRD) in multiple myeloma and an evident correlation between the entity of molecular positivity measured in leukapheresis or in bone marrow after transplant and clinical outcome does not exist. Peripheral blood stem cell transplantation offers about 50% of clinical complete remissions in this malignancy, but a lot of patients relapse and the significance of their molecular status should be better clarified. In harvest products a reduction of the IgH clonal rearrangement after high-dose therapy was found. Moreover, 20-40% of CR has been reported after single/double PBSCT, but only 7% of cases achieved molecular remission. Allogeneic transplantation, perhaps because of its graft-versus myeloma effect, seems to offer a higher remission rate, with 50% of PCR-negativities. This group of patients could achieve molecular remission also after 5-6 years and an evident correlation between PCR status and RFS has been reported. In this study, fifteen patients were molecularly evaluated by two quantitative methods: GeneScan and real-time PCR. At diagnosis, the entity of the disease molecularly evaluated did not correlate with biological variables (bone marrow infiltration percentage, β2-microglobulin, TK, PCR levels, number of osteolytic areas). Both methods showed PCR-positivity in harvests from all patients treated by high-dose therapy, but a reduction of the IgH clonal rearrangement of 1-3 log was described in all cases. After autotransplant, 17% of molecular remissions were found, with a patient achieving the PCR-negativity only after the second graft. All patients still PCR-positive after transplant were already positive at diagnosis and received contaminated precursors. Eight patients have now a median follow up of 24 months: in 6 of them a progression of the malignancy occurred. This progression was always predicted by a quantitative increase of the IgH rearrangement showed by both molecular assays. Thus, results reported above confirm the important role of MRD quantitative molecular monitoring even for patients affected by multiple myeloma.

**PO212**

**EMERGENCE OF A NEW CLONAL ABNORMALITY IN CYTOGENETIC AND HEMATOLOGIC COMPLETE REMISSION IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA**


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Chronic myeloid leukemia (CML) is characterized by the presence, in more than 90% of patients, of Philadelphia chromosome (Ph). The treatment of CML with interferon-α has resulted in complete hematologic response (CHR) rates of 70 to 80%, and cytogenetic response rates of 50% which are major and durable in 25 to 30%. The latter patients have had the most favorable outcome with IFN-α therapy. We report a case of a patient with Ph+ CML who achieved complete cytogenetic remission (CCR) and developed new cytogenetic Ph- clone. A 61-year-old male was diagnosed to have CML in June 1997. At diagnosis, cytogenetic analyses showed only t(9;22)(q34;q11) in all bone marrow metaphases and reverse transcription polymerase chain reaction (RT/PCR) study for BCR/ABL rearrangement showed aβ transcript. After 29 months of IFN-α therapy the cytogenetic studies on bone marrow aspirate showed 33.3% Ph-positive metaphases and the other cells (66.7%) had a new cytogenetic abnormality, t(2;11)(p21;q23), without Ph chromosome. His constitutional karyotype from peripheral blood was normal. Because of intolerance to IFN-α therapy, the patient was enrolled in new protocol of therapy based on the dispersion of STI-571. After 8 months he achieved complete hematological and cytogenetic remission with a karyotype 46,XY, t(2;11)(p21;q23) in all analyzed cells. In addition to standard G-banded metaphases analysis, we performed fluorescent in situ hybridization (FISH) techniques using a BCR/ABL1 D-FISH translocation DNA probe (Appligene Oncor) in order to verify the presence of t(9;22)(q34;q11). We observed 434 cells and 2 cells with Ph abnormality were found. In addition, FISH with a LSI MLL Dual Color probe (Vysis, Inc.) in order to evaluate MLL rearrangement was negative. RT/PCR for BCR/ABL was weakly positive and quantitative PCR has been performed during the course of therapy. The patient is well and in Ph cytogenetic remission. This patient developed a new cytogenetic abnormality during achievement of the CCR with IFN. A few similar cases are described with different cytogenetic abnormalities and in most of the patients the emergence of new clonal abnormalities is associated with secondary hematological disorders. These changes can be due to the effect of therapy or the selective Ph suppression obtained with IFN-α therapy may allow the evolution and the expansion of other suppressed clones existing simultaneously or arising in time.
marrow cells, peripheral blood stem cells and cord blood cells. Granulocyte-macrophage colony-forming units (GM-CFU) were significantly increased in all sOPG10-14-treated samples, whereas granulocyte-erythroid-monocyte-megakaryocyte CFU (GEM-M-CFU) and burst-forming unit erythroid (BFU-E) were increased only in the cord blood test. Moreover, sOPG10-14 preserves the self-renewal potential of stem cells in long-term culture-initiating cells (LTC-IC), without inducing terminal differentiation or exhaustion of pluripotent stem cells. Preliminary experiments suggest that sOPG10-14 is able to act directly on CD34+ enriched cells. The pentapeptide activity is mediated by an increased activity of stem cell factor (SCF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) as proven by experiments on factor(s) dependent cell lines. A highly complex network of cytokines regulates the hemopoiesis and OGP10-14 seems to take part in this process.

PO234
"JUMPING" TRANSLOCATIONS OF 1q IN ACUTE LYMPHOBlastic LEUKEMIA

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Jumping translocations (JTs) are rare translocations involving a single donor chromosome and two or more recipient chromosomes. Jumping translocations have been primarily described as acquired changes observed in tumor cell populations of hematologic malignancies, mainly lymphoid disorders such as acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma and multiple myeloma. Their presence in a tumor cell population is a marker of aggressive disease. These aberrations have also been reported as constitutional changes involving the long arms of chromosome 15 and occurring in patients with Prader-Willi syndrome. The preponderance of chromosome 1 involvement as JTs donor chromosome in acquired aberrations initially suggested it was non-randomly involved; on the other hand recipient chromosome involvement appears random in terms of chromosome identification, but with an apparent preferential involvement of the distal/telomeric regions. We report a case of ALL-L3 in which JTs of the long arms of chromosome 1 were detected. At diagnosis chromosome analysis has been performed in 32 metaphases: only one was normal. All tumor cell population showed t(8;14)(q24;q32) confirming the diagnosis of L3-ALL. The JTs breakpoint on donor chromosome 1 was localized at 1q21. In all abnormal cells, a JT involving 1q32, der(1)t(1;1)(q21;q32), was present. In addition, other telomeric segments are involved in the JTs. The recipient chromosome were as follows: 22p (8 metaphases), 15p (2 metaphases), 4q (2 metaphases), 11q (2 metaphases) and 19p (2 metaphases). In the 8 remaining cells JTs were non-clonal. The patient was treated; but she did not achieve complete remission of the disease and the cytogenetic analysis showed abnormal karyotype in 16 out of 30 observed metaphases. In this occasion, the JTs involved, as recipient, 1q32 (in all tumor cell population) and 4q35 (in 11 metaphases); in the 5 remaining cells the JTs involved 5 different chromosomes. This karyotype could be indicate a decreased breakpoint complexity and a clonal selection. In addition to standard G-band-ed metaphase analysis, we are examining these JTs by FISH techniques using telomeric sequence (T2ZAG3In) DNA probes and multiple whole chromosome DNA painting probes for the involved recipient chromosomes and the donor chromosome and by molecular studies, too. This patient can be an additional case to support the belief that JTs of the long arm of chromosome 1 correlate with an aggressive disease. Although the belief most of the cases of JTs previously reported are associated with a poor clinical outcome, their prognostic impact is difficult to assess given the few cases described.

PO215
B-CELL PRECURSOR BONE MARROW RECONSTITUTION AFTER AUTOLOGOUS AND ALLOGENIC TRANSPLANTATION OR CONVENTIONAL CHEMOTHERAPY IN ACUTE LEUKEMIA

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Hematogones are an important finding in the marrow of both children and adults after autologous and allogeneic transplantation or conventional chemotherapy for acute leukemia. These cells appear to be independent of age and have a normal precursor B-cell surface antigenic phenotype (CD19+, CD10-, CD22+, CD20 negative to dim) and generally express very little CD34. The clinical significance of hematogones has not been resolved, and the biologic features of these cells are incompletely described. In some patients, these cells may account to greater than 50% of the bone marrow cells, creating a picture that can be confused with acute lymphoblastic leukemia (ALL) or metastic tumor. In this study we correlated the clinical and flow cytometric properties of hematogones evaluating the bone marrow samples from 55 AML, 22 ALL-B, 6 ALL-T patients, using four-colour and CD45-gating strategies and different combinations of antibodies: CD10 FITC/CD20 PE/CD45 PerCP/CD19 APC; HLA-DR FITC/CD22 PE/CD45 PerCP/CD19 APC; CD10 FITC/CD34 PE/CD45 PerCP/CD19 APC. We found hematogones in regenerating marrow of 25 AML (45.5%), 9 ALL-B (40.9%) and 3 ALL-T (50%) patients with absence of cytologic atypia or abnormal localization of lymphoid cells in the BM biopsy; normal BM karyotype and no evidence of neoplastic marrow involvement, confirmed by clinical follow-up. These subpopulations were not found to be therapy correlated and age related. Two immature CD19− subpopulations co-expressing B-cell associated antigens were identified: CD34−/CD19−/CD22+/CD10−/HLA DR− and CD34−/CD19−/CD22+/CD10−/HLA DR− (the greatest subset) with different SSC/CD45 distribution on the cytogram, never merging with the ALL-B blast region. Regarding these last two characteristics, it was possible to distinguish the normal B-precursors from the neoplastic cells in regenerating marrow of ALL-B patients in the immunological detection of minimal residual disease. In fact, in our experience, the ALL-B blasts, both as diagnosis and relapse, had a different SSC/CD45 distribution than the hematogones. These normal B-precursors were found only during remission status, instead they were missing at the relapse. Therefore the significance of this, being unknown, should be more investigated to confirm or not any relation with acute leukemia relapse, especially with ALL-B relapse.
### PO216
**MINIMAL RESIDUAL DISEASE MONITORING IN NON-HODGKIN'S LYMPHOMA**

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Seventy-two patients affected by non-Hodgkin's lymphoma (equally distributed for indolent and aggressive histotype) were evaluated for the presence of a detectable molecular marker (IgH, bcl-1, bcl-2 rearrangement) at diagnosis, after high-dose chemotherapy on harvests and after peripheral blood stem cell transplantation in order to find a possible predictive role of the minimal residual disease on the treatment outcome. In the entire series overall response rate was 72%, with 62% of complete remissions. Histology, stage of neoplasia and molecular status at diagnosis did not significantly correlated with clinical outcome. Fifty-three patients (88%) are alive after a median follow up of 60 months. Five-year OS was 87%, better for indolent than aggressive lymphomas; RFS was 73% for aggressive and 62% for indolent histotypes. At diagnosis, 59% of patients, more frequently indolent and advanced cases, showed a detectable molecular marker. The molecular status at diagnosis did not condition clinical outcome. After 6 CHOP-like courses and high-dose therapies, harvests from 62% of patients resulted still PCR-positive, without differences between indolent and aggressive cases. Molecular remission rate was 18%. Interestingly, 31% of patients PCR-negative in the bone marrow at diagnosis mobilised PCR-positive precursors. The ex vivo CD34+/CD19+ purging offered only minimal advantages in the relapse rate (22% of patients receiving purged precursors relapsed versus 32% of cases receiving unpurged graft). Nevertheless, OS rate resulted higher for patients transplanted with unpurged stem cells. After PBSCT, 65% of patients resulted PCR-negative, with 50% of molecular remissions; the relapse rate was 55% for patients still PCR-positive after transplant versus 29% for those PCR-negative. OS and RFS rates resulted higher in the group of patients PCR-negative after transplant. Molecular quantitative than qualitative assays for the evaluation of the minimal residual disease could have a further significant prognostic value.

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**A good correlation was observed between flow cytometric differential and morphological analysis of BM aspirates. The mean percentage of CD34+ cells and the distribution of lymphocyte subpopulations (T, NK and B) were easily obtained. The high standard deviation observed for some parameters was essentially due to the variability in total cell count that depends on the magnitude of hemodilution. The study of a larger number of cases will allow stratification of samples in relationship with the total cell count, thereby permitting to better define reference values for flow cytometric analysis of BM aspirates.**

### PO217
**REFERENCE VALUES OF BONE MARROW LEUKOCYTE POPULATIONS: A FLOW CYTOMETRIC STUDY**

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Flow cytometric analysis of bone marrow (BM) aspirates is central to diagnosis, staging and follow up of hematological malignancies. However, no data are available in the literature about reference values of cellular populations obtained by immunophenotypic flow cytometry. Furthermore, many variables may influence percentages and ratios between cell populations (i.e. sample drawing, age, chemotherapy). Thus, in spite of intrinsic difficulties, a flow cytometric classification on bone marrow aspirates requires of reference values. For this purpose four laboratories (belonging to GPMI) have defined a protocol to study BM aspirates. The criteria for pre-analytical and analytical procedures as well as the monoclonal antibodies panel (10 three color stainings) were strictly defined. The aim of the study was to define: a) a flow cytometric BM differential, b) a quantitative distribution of cell compartments of myeloid differentiation, c) a quantitative distribution of lymphocyte subpopulations. Gating strategies and analytical approach were also standardized to allow a complete comparison of data obtained from the four laboratories. The samples included in this study were from staging of lymphoma (negative for BM involvement both immunophenotypically and molecularly), idiopathic thrombocytopenia or other disorders with no known alterations of BM populations.

Results obtained from 108 samples (58 F, 50 M) are reported in the table:

<table>
<thead>
<tr>
<th>Mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>Cell count</td>
<td>24.2</td>
</tr>
<tr>
<td>Whole lymphoid serie</td>
<td>11.7</td>
</tr>
<tr>
<td>Erythroid serie</td>
<td>5.8</td>
</tr>
<tr>
<td>Monocytes</td>
<td>14</td>
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<tr>
<td>Erythrocytes</td>
<td>27.2</td>
</tr>
<tr>
<td>Whole myeloid serie</td>
<td>14</td>
</tr>
<tr>
<td>Immature</td>
<td>18.3</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>42.2</td>
</tr>
<tr>
<td>Myeloid metamyelocytes</td>
<td>35.6</td>
</tr>
<tr>
<td>Segmented</td>
<td>50.5</td>
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</tbody>
</table>

A good correlation was observed between flow cytometric differential and morphological analysis of BM aspirates. The mean percentage of CD34+ cells and the distribution of lymphocyte subpopulations (T, NK and B) were easily obtained. The high standard deviation observed for some parameters was essentially due to the variability in total cell count that depends on the magnitude of hemodilution. The study of a larger number of cases will allow stratification of samples in relationship with the total cell count, thereby permitting to better define reference values for flow cytometric analysis of BM aspirates.

### PO218
**SERUM THROMBOPOIETIN AND ERYTHROPOIETIN LEVELS IN CORD-BLOOD, CHILDREN, HEALTHY ADULT VOLUNTEERS AND PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA**

B. Feola,*, S. M isso,*, G. Fratellanza,*, V. M inerva,*, P. Concilio,*, A. M inerva,*

*Servizio di Immunoematologia e Medicina Trasfusionale A. O. Caserta, °Servizio di Immunoematologia e Medicina Trasfusionale A. U. P. Federico II Napoli

Thrombopoietin (TPO) is a glycoprotein that primarily regulates megakaryocyte development and platelet production. Elevations of endogenous TPO values have been observed in disorders

<table>
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with reduced platelet/megakaryocyte mass, including aplastic anemia and chemotherapy-induced thrombocytopenia. Increased TPO levels precede thrombocytosis in an inflammatory disorder. These effects of TPO in thrombopoiesis are similar to the key roles played by erythropoietin (EPO) in erythropoiesis. The changes in blood TPO levels throughout child development remain unknown, in contrast to extensive studies on endogenous levels of EPO. In this study we measured TPO and EPO levels in 102 serum sample. We tested 32 serum sample obtained from cord-blood, 30 from disease-free children (age 6–10 years), 25 from healthy adult (age 31–47 years) volunteers and 15 patients with essential thrombocythemia (ET) untreated. TPO and EPO concentrations in serum were measured by sensitive ELISA. Results were expressed as means±SD; differences were considered significant when the two-tailed p value was <0.05. Results are shown in the table below.

<table>
<thead>
<tr>
<th>TPO</th>
<th>EPO</th>
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<tr>
<td>Cord-blood</td>
<td>31.5±26</td>
</tr>
<tr>
<td>Children</td>
<td>16.1±7</td>
</tr>
<tr>
<td>Donors</td>
<td>6.7±6</td>
</tr>
<tr>
<td>Essential thrombocythemia</td>
<td>8.4±3</td>
</tr>
</tbody>
</table>

Discussion and Conclusions. Mean TPO serum levels were significantly higher in children than adult (p<0.05). Cord-blood contained higher levels of TPO. In contrast, mean EPO serum levels were slightly higher in cord-blood than children (p<0.05). In adult EPO serum levels were decreased. Serum EPO levels in ET untreated patients were lower compared with serum EPO levels in healthy control patients, children and cord-blood (p<0.05); while serum TPO levels for untreated ET were higher than serum TPO levels of the healthy controls but were lower than children and cord-blood (p<0.05). The results of our study suggest a difference in the regulation of serum TPO in patient with ET. The exact mechanism of elevated TPO levels during the neonatal period remains under investigation. The elevation of blood TPO levels might be explained by an increased rate of TPO production, decreased TPO clearance, and/or reduced c-Mpl mass on megakaryocytes/platelets. To clarify the physiological regulation of thrombopoiesis in infancy and children, further studies on the c-Mpl and TPO system should be conducted.

PO219
AUTOMATIC ENUMERATION OF RETICULOCYTES: ANALYSIS OF THE RELATIONSHIPS BETWEEN DIFFERENT PARAMETERS


Reticulocyte count (R.C.) plays a central role in monitoring both primary and secondary anemia, in particular in cancer and uremic patients. In addition, RC has been recently used for the control of blood doping procedures in endurance athletes. The present availability of new and automated instruments for RC has significantly enhanced both the precision and the reproducibility of the assay. In particular, a number of new parameters, which include reticulocyte volume and different maturation indices, are at present available. While no doubts exist on the clinical relevance of RC, other parameters require an on field validation before introduction in the clinical monitoring of erythroid maturation studies. For these reasons, we have analyzed the differences between RC obtained using different technical approaches and the relationships between RC and other reticulocyte-related parameters. The aim of the study was to define the statistical dependence/independence of reticulocyte-associated parameters. Thus, R.C. have been performed using a reticulocyte counter (R-3000, Dasit), an automated hemocytometer (GEN-S) and a flow cytometer EPICS XL (both from Beckman Coulter, IL, Milan, Italy). The number of samples to be studies was calculated on the basis of expected RC results. All samples were analyzed within six hours from bleeding. As expected, the relationships between different instruments were statistically high but the reproducibility index (calculated according to Bland and Altman, 1986) was better in automated counters than in the flow cytometer. The analysis of the relationships between R.C. (obtained from the reticulocyte counter and the flow cytometer) and novel parameters (such as reticulocyte volume and immature reticulocyte indices, obtained by the GEN-S) clearly indicated the independence between reticulocyte-associated parameters and RC. In particular, the presence of high numbers of reticulocytes did not significantly correlate with large volumes and evident indices of circulating immature reticulocytes. On these bases, the presence of a stimulated maturation/mobilization of cells belonging to the erythroid lineage can be supposed not only in the presence of high percentages of circulating reticulocytes, but also by detecting the presence of either immature or large volume erythrocyte precursors.

PO220
RAPID IDENTIFICATION OF CYTOGENETIC ABNORMALITIES BY PRIMED IN SITU HYBRIDIZATION

L. Luciano, F. Frigeri,* M. Barone, G. Iaccarino,* A. Pinto,* B. Rotoli Cattedra di Ematologia, Federico II University; *Hematology-Oncology Unit, National Cancer Institute "G. Pascale", Naples

Leukemias and lymphomas show cytogenetic abnormalities (chromosome translocations, deletions, trisomies, polysomies) useful for diagnosis and during the follow-up. Conventional chromosome analysis shows very high specificity, but it analyzes only proliferating cells. Introduction of molecular cytogenetic techniques such as fluorescent in situ hybridization (FISH) has improved the detection of both numerical and structural chromosome abnormalities, being more sensitive. Recently, a faster alternative to FISH with the same sensitivity, specificity and accuracy is primed in situ labeling (PRINS), a method for labelling specific DNA sequences by annealing an oligonucleotide DNA primer to the denaturated DNA on glass slide and extending it enzymatically in situ with the incorporation of labelled nucleotides. We set up this technique for detecting and monitoring sex mismatched alloBMT recipients using X and Y primers on cell pellet for cytogenetic analysis and on bone marrow or peripheral blood smears. The results were then compared to results of FISH and chromosome analysis. We also utilized this technique to detect aneuploidy of chromosome 8 in three cases of acute myeloid leukemia (two cases with trisomy 8, one with t(n15) by FISH, but faster and cheaper.
PO221
FLUORESCENT IN SITU HYBRIDIZATION CHARACTERIZATION OF 3P INVOLVEMENT IN COMPLEX KARYOTYPES OF ACUTE MYELOID LEUKEMIA

C. Nozzoli, R. La Starza,* S. Colli, F. Leoni, M. Piazziini,* S. Frontera,* A. Bosi, P. Rossi Ferrini, C. Mecucci*
Department of Hematology, University of Florence, *Hematology, University of Perugia

Rearrangements of the short arm of chromosome 3 (3p) are recurrent changes in breast, head, neck, lung and kidney carcinomas. In hematological malignancies the incidence of 3p abnormalities varies from 1 to 3% in lymphoid and myeloid disorders, respectively. High frequency of 3p involvement has been reported in therapy-related acute myeloid leukemia (AML). We collected four AML cases with a complex karyotype including a 3p aberration. All patients were males. Median age was 65. Past history revealed a possible professional exposure in three cases. In one of them plus an additional case, the leukemia was preceded by refractory anemia with excess of blasts. Moreover in all cases but one bone marrow dysplasia was detectable at time of leukemia infiltration. A concomitant colon carcinoma was diagnosed at the time of acute leukemia (AL) onset in one case. All four patients underwent standard induction chemotherapy. Two patients died during induction therapy, one died in complete remission (CR) and one did not respond. Cytogenetically, 3p abnormalities were represented by deletion (one case), unbalanced translocations (two cases), and reciprocal translocation (one case). Deletion or monosomy of chromosome 5 were present in all cases. One case showed monosomy 7, and two cases trisomy 8. The nature of complex changes was investigated using paintings for chromosomes 2, 3, 5, 6, 7, 12, 14, 20, and 22 in green, red and orange (Vysis, Oncor). Paintings revealed unexpected results for a number of structural karyotypic aberrations. Concerning 3p involvement, loss of 3p material was confirmed in the patient with cytogenetic evidence of deletion. In the two cases with unbalanced translocations loss of material from 3p was also proved and paintings 5 and 12 identified the partners of translocations. In the case with the t(2;3)(q36;p12) a reciprocal exchange was confirmed by painting 2 and 3. Results from this series of AML with 3p abnormalities strongly support the secondary nature of the associated hematological disorders. Cytogenetically, all cases showed a complex karyotype and involvement of chromosome 5. Moreover, bone marrow dysplasia and/or preceding history of professional exposure were present in all patients. From this study it was also evident that loss of material from 3p is the predominant genomic event underlying different rearrangements, suggesting a critical involvement of tumor suppressor genes in the pathogenesis of secondary AML with a very bad prognosis. As far as we know the association between colon carcinoma and AML with 3p aberrations has never been described.

PO222
DESCRIPTION OF A CRYPTIC t(15;17) IN A PATIENT WITH ACUTE PROMYELOCYTIC LEUKEMIA WITH PML/RARα REARRANGEMENT ON CHROMOSOME 17

A. Zaccaria,* M. Toschi, A. Valenti,* M. Salvucci, R. Cipriani, E. Ottaviani,* G. Martinielli*

Acute promyelocytic leukemia (APL) almost always involves a chromosomal reciprocal translocation t(15;17)(q22;q21) that results in the fusion of retinoic acid receptor α (RARα) gene with a transcription factor called promyelocytic leukemia (PML) gene. Moreover several cases of APL with t(11;17) and t(5;17) variant translocations have been described, involving fusion of the RARα gene with the promyelocytic leukaemia zinc finger (PLZF) gene on chromosome 11 and nucleophosmin (NPM) on chromosome 5 respectively. An isochromosome for the long arm of chromosome 17 (i17q) has been reported as an indicator of a poor prognosis, but its clinical significance is still unknown [1, 2]. A new patient with acute promyelocytic leukemia was observed with a cryptic t(15;17) and a good clinical response. The karyotype was 47,XY,i(17q)del(5)(p15.3). FISH analysis was performed utilizing Vysis probes: a 180 kb one labeled with Spectrum Orange fluorophore for PML and a 400 kb one labelled with Spectrum Green fluorophore for RARα. Usually, the PMLRARα rearrangement occurs on chromosome 17, both in the cases characterized by the t(15;17) and in those with cryptic variants. Surprisingly, we detected two strong red (PML) signals on chromosomes 15 and two green (RARα) ones on chromosomes 17. Moreover, in a high percentage of the cells a very small PML signal was detected on a chromosome 17, overlapping the green (RARα) one. In the literature, PMLRARα fusion genes occurring on chromosome 17 have been rarely described. When present, they are associated with gross, cytogenetically detectable translocation of chromosome material from chromosome 15 to chromosome 17. In one case only, previously reported, chromosome 15 and 17 were morphologically normal, but other anomalies were present in the karyotype, even not involved in the PMLRARα-translocation. To our knowledge, this is the first case of APL in which the molecular rearrangement has been located on chromosome 17 in the context of a normal karyotype.

of the derivative 17 (ider(17q)t(15;17)) has been observed only in a few cases of APL. Here we describe two additional cases of APL whom cytogenetic analysis on bone marrow cells at diagnosis showed a 15q+ and a derivative chromosome 17 (isochromosome-like) as sole anomaly in almost all observed Q-banded metaphases. We employed fluorescent in situ hybridization (FISH) with a chromosome painting probe for chromosome 15 (Appiglen-Oncor Total Chromosome Paint 15) and a centromeric probe for chromosome 17 (Appiglen-Oncor 15α satellite 17 Probe), which confirmed that the isochromosome consisted of two long arms of the der(17)t(15;17). Moreover the two fluorescent spots or the larger size of fluorescent spot at centromeric region showed the ide(17) as a dicentric chromosome. This supports recent findings which have proved by FISH that a large number of isochromosomes identified by chromosome banding analysis actually are isodicentric chromosomes. FISH with dual-labeled PML and RARα probes (LSI PML/RARα dual Vysis-Olympus Probe) demonstrated the presence of the fusion gene PML/RARα on the derivative chromosome 15+. Our results with FISH represent a further confirmation of previous interpretations on the ide(17q) found in APL based above all on conventional cytogenetic techniques.

PO224
PERI-LESIONAL INJECTIONS OF GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR IN THE MANAGEMENT OF CHRONIC LEG ULCERS IN PATIENTS WITH HEMATOLOGIC DISEASES

R. Bertè, D. Vaillisa, A. Lazzaro, C.F. Moroni, G. Civardi, L. Cavana
Department of Internal Medicine and Onco-Hematology, Ospedale Civile di Piacenza, Piacenza

Peri-lesional injections of recombinant granulocyte-macrophage colony-stimulating factor (rhGM-CSF) improved the healing of biopsy wounds in 35 patients with leprosy and induced the closure of Kasopi’s sarcoma lesions in one patient; beneficial effects were obtained also in chronic leg ulcers of patients with hemoglobinopathies. We report the effects of local injection of rhGM-CSF (Mielogen-Molgrastim; Schering-Plough) in three patients with hepatitis C virus (HCV)-related type II cryoglobulinemia with non-Hodgkin’s lymphoma and one patient with polycythemia vera, in which chronic leg ulcers would not heal despite the wide variety of treatments applied. Patient #1 was a 65-year old woman who had had a chronic painful ulcer on the left ankle (3.2x2.7 cm) for 6 months. Patient #2 was a 62-year old woman who had had two deep ulcers, one on her left ankle (3.7x3.2 cm) and one on the dorsum of the left foot (2.5x2.2 cm), for 10 months. Patient #3 was a 63-year-old woman who had had two deep ulcers on his right leg (2.5x1.5 cm and 1.5x1.3 cm) for the past 3 years. All these patients had HCV-related type II cryoglobulins with non-Hodgkin’s lymphoma: diffuse large cell lymphoma in complete remission (CR), gastric MALT lymphoma in CR, immunocytoma in partial remission. The three patients had an IgM-κ paraprotein which behaved like a cryoglobulin. Patient #4 was a 73-year old woman who had had a chronic painful ulcer on the right leg (7.4x4 cm). These patients had polycythemia vera. GM-CSF (Mielogen-Molgrastim; Schering-Plough) 300 mg was injected subcutaneously into four sites within the margins of the wounds, in approximately equal amounts, in the four quadrants of each ulcer, through a insulin syringe needle, twice a week for 2 months. In some instances, a small quantity of the solution was applied over the open ulcer. The injections were quite painful, but were well tolerated in all 4 patients; no other side effects were recorded and complete resolution of the ulcers was seen. In the first and second patients, the ulcers healed after 6 weeks; the ulcers of patient #3 required approximately 2 months to heal completely; the ulcer of patient #4 healed after 9 weeks. We believe that subcutaneous perilesional injection of GM-CSF may play an important role in the cure of chronic leg ulcers in patients with hematologic diseases.

PO225
CLINICAL SIGNIFICANCE OF DEL(20q) IN HEMATOLOGIC MALIGNANCIES

A. Tedeschi, M. Montillo, K.J. Hayes,* F. Di Raimondo,* S. Lerner,* E. Morra, E. Estey,* M.J. Keating*
*Leukemia Unit, University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; °Chair of Hematology, “Ferrarotto” Hospital, Catania; Department of Hematology, Niguarda Ca’ Granda Hospital, Milan

The del(20q) is the second most common primary clonal structural abnormality seen in hematologic disorders behind the Philadelphia chromosome. The M. D. Anderson Cancer Center records were searched to identify pts presenting with the del(20q). The del(20q) was identified in 126 pts, in 46.4% the deletion was the sole cytogenetic abnormality. Clinical diagnosis was MDS in 46.8% cases, AL in 22%, CMD in 17.5%, 14 pts presented with lymphoproliferative disorders, 2 with multiple myeloma and 1 with aplastic anemia. The 59 MDS pts were distributed as follows: 44.1% RA, 13.6% RARS, 15.2% RAEB, 25.4% RAEB-t, 1.7% CMML. The association of a complex karyotype was higher in pts with RARS, RAEB and RAEBt compared with in pts with RA (p<0.01). Overall 38.6% pts developed AML, time to progression was 24.5 months for RA pts and 5 months for RAEB or RAEBt pts. The median survival was: 30 months for RA and RARS pts and 10 months for pts with RAEB or CMML (p<0.001). No difference in survival was observed between the two groups when only pts with isolated del(20q) were considered; a complex karyotype conferred a shorter survival to pts with advanced MDS (p<0.004). Seventeen out of the 28 pts with AL presented with AML or AUL, median age was 62 years, in 6 pts the del(20q) was the sole cytogenetic abnormality; two pts presented with a secondary AML. A CR rate of 70.5% was achieved after induction therapy, all but one pts relapsed; the survival of pts with AML and AUL was not significantly different when compared to pts with RAEB and RAEBt. Unlike pts with advanced MDS, pts. with AML and AUL showing only the del(20q) did not have a better survival in respect of the group with a complex karyotype. There were 11 pts with ALL, 10 with B-ALL, median age 41.6 years. Seven pts presented a complex karyotype, in only two cases was there an association with Philadelphia chromosome. A low CR (54%) rate was observed after induction therapy all but one pts relapsed; the survival of pts with AML and AUL was not significantly different when compared to pts with RAEB and RAEBt. Unlike pts with advanced MDS, pts. with AML and AUL showing only the del(20q) did not have a better survival in respect of the group with a complex karyotype. There were 11 pts with ALL, 10 with B-ALL, median age 41.6 years. Seven pts presented a complex karyotype, in only two cases was there an association with Philadelphia chromosome. A low CR (54%) rate was observed after induction therapy all but one pts relapsed; the survival of pts with AML and AUL was not significantly different when compared to pts with RAEB and RAEBt.
PO226
PERICENTRIC INVERSION OF CHROMOSOME 9 IN MYELOPROLIFERATIVE AND MYELODYSPLASTIC SYNDROMES

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Cattedra di Ematologia, Università di Parma

Pericentric inversion of chromosome 9 \(\text{inv}(9)(p11;q13)\) represents a minor chromosomal rearrangement which does not correlate with abnormal phenotypes. Its incidence has been reported to be about 1% in the general population. The pathogenetic significance of \(\text{inv}(9)\) is still an enigma and results of investigations are controversial. Several reports have shown an increased incidence of \(\text{inv}(9)\) in patients with different solid tumors and hematologic diseases as compared with healthy controls. Other authors, however, have found conflicting results. Here we report data on \(\text{inv}(9)\) incidence in a group of 110 patients, 43 of whom with CML and 67 with MDS, in order to establish a possible correlation between this heterochromatic variant and the considered pathologies. In our patients we observed an \(\text{inv}(9)\) incidence of 5.45%, which value is higher than the general population one (1%). However we did not find a statistically significant increase of \(\text{inv}(9)\) incidence \((p>0.05)\), comparing our patients with healthy controls, even if difference was large. In 3 of our CML patients \(\text{inv}(9)\) was associated to \(t(9;22)(q34;q11):\) in all 3 cases the deleted material from chromosome 22 was found to be translocated to the homolog of chromosome 9 without pericentric inversion, so showing a non random translocation event, contrary to most of reports. Moreover the \(t(9;22)\) was always observed on the same homolog in all analyzed metaphases, supporting the theory of clonal evolution of leukemic cells. In conclusion, although our results have not shown a statistically significant increase of \(\text{inv}(9)\) incidence in hematologic patients, it seems important to point out that this and other pericentric rearrangements \((\text{es. inv}(1), \text{dic}(9;12)(p12;p11), \text{dic}(17;18)(p11;p11))\) appear to occur more frequently than expected in malignant cells. So it is reasonable to suppose that they may have a prominent role in developing neoplasia, producing chromosomal instability.

PO227
MYELOPEROXIDASE ABNORMALITIES

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Many automatic analyzers give the clinicians the possibility of evaluating differential blood count exploiting perox cytochemistry combined with cell volume measurement. We have studied 36,000 peripheral blood samples of in- or out-patients, and 3,000 controls whose MPXI (mean peroxidase value) was between \(+10\) and \(-10\). Each sample was evaluated by the H3-Bayer autoanalyzer, which exploits perox cytochemistry. MPXI value allowed the allocation of each sample into one of three groups: a) \(\text{MPXI} < -10\); b) \(\text{MPXI} \approx -10\) and \(+10\); c) \(\text{MPXI} > +10\). Samples coming from subjects who were not affected by acute or chronic diseases showed MPXI values \(\approx 0\). We identified 1957 patients with myeloperoxidase deficiency (D-MPO), 648 with MPXI increase and 33,395 with normal MPXI. Two hundred cases showing abnormal MPXI were also evaluated by cytochemical staining of blood smears, and the results were in agreement with the automatic evaluation. D-MPO patients had M/F and adult/children ratios of 1.32 and 7.33, respectively. Only 12 were completely enzyme deficient: this group was composed by pregnant women, kidney transplanted patients taking cyclosporin-A, dialised patients, patients affected by liver diseases or by cancer, anemia or dyslipidemia. Patients showing partial D-MPO were affected by various diseases, including the above mentioned: 68% of cases had neutrophil and 32% had eosinophil MPO deficiency. We did not find associations between the entity of D-MPO and diseases, although it is known that this condition can impair anti-infective defenses.
with short survival. We report a case of trisomy 8 associated with acute myeloid leukemia M5 as sole cytogenetic abnormality. At diagnosis, the patient showed the following karyotype: 47 XY +8 (80%) /50 XY +8+8+8+8 (20%). FISH analysis and primed in situ labeling (PRINS), that analyzes even interphase cells, showed a higher percentage of trisomy 8 cells (30%). The patient was treated with the GIMEMA protocol AML 99P and obtained clinical and cytogenetic remission. Since a HLA identical sister was available, the patient was addressed to allogeneic bone marrow transplantation. At the time of BMT, the patient was in leukemia relapse, confirmed by FISH and PRINS analysis that showed the same pattern as at diagnosis. After engraftment, assessed by FISH and PRINS analysis (sex switch), the patient received DLI infusion; at +7 months he is still alive in complete remission. All cases of chromosome 8 polysomies reported so far had FAB M5 phenotype and showed very short survival. To our knowledge, this is the only case successfully treated by BMT. After transplant, we decided to elicitate an immunologic response (GvL) by DLI, assuming that these cells may be more sensitive to the GvL effect because of overexpression of surface antigens, due to the polysomy.

Rituximab and Campath-1H are unconjugated humanized IgG1 monoclonal antibodies that recognize the CD20 and CD52 antigens. They are being proposed for the treatment of follicular and other B-NHL (rituximab) and of B-CLL (Campath-1H). Their mechanism of action is thought to include antibody dependent cellular cytotoxicity (ADCC) and complement mediated cytotoxicity (CDC). The capacity of both antibodies to induce ADCC and CDC has been studied nearly exclusively on B cell lines and they have never been directly compared with each other. We have investigated the lysis of several different freshly isolated cases B-CLL or mantle cell lymphomas induced by either Rituximab or Campath-1H and through both CDC using the Alamar blue test and ADCC using a human NK cell line (NKL) as effector cells. In addition we have measured their capacity to lyse 3 different B lymphoma cell lines. Finally we have related our findings to the levels of expression of the target molecules, CD20 and CD52, on the different cells. Both rituximab and campath-1H induced efficient lysis of the different cell lines through ADCC, reaching 60-80%. On the contrary, only campath-1H antibody was able to induce ADCC in fresh leukemic samples, reaching 40-70%, rituximab being virtually inactive. Similarly campath-1H was shown to induce very strong CDC (80-99%) on all CLL and MCL samples tested, whereas rituximab was in several cases less active (1-90%). The particularly high killing capacity of campath-1H relative to rituximab appears to be at least in part due to higher expression levels of the CD52 molecules on fresh leukemic samples relative to CD20, a phenomenon not observed on the B cell lines tested where in fact both MAbs showed similar activity. A more precise quantitative evaluation of these molecules using calibrated beads will be presented which may support the use of such determinations to predict the in vitro and potentially in vivo response of different patients to these therapeutic MAbs.

**PO230**

**CAMPATH-1H MEDIATES ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY AND COMPLEMENT MEDIATED CYTOTOXICITY MORE EFFICIENTLY THAN RITUXIMAB ON FRESHLY ISOLATED LEUKEMIC B CELLS**

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**PO231**

**THE EXPRESSION OF CD10 BY B-CELLS OF CHRONIC LYMPHOCYTIC LEUKEMIA IS RELATED TO APOPTOSIS**

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In the present study, we investigated whether the relationship between CD10 expression and apoptosis is also demonstrable for B-CLL cells. We analyzed 10 previously untreated B-chronic lymphocytic leukemia (B-CLL) cases for CD10, annexin-V expression, and PI staining in the presence of various stimuli. An increased expression of CD10 was found in B-CLL cells undergoing apoptosis in vitro, either spontaneously or drug-induced or through signals delivered by surface IgM cross-linking. In particular, CD10 appeared to be expressed early during the apoptotic process, soon after phosphatidylserinere externalization and before DNA fragmentation. In addition, VAD-fmk prevented etoposide-induced expression of surface CD10 other than nuclei fragmentation. Moreover, to demonstrate the finding that CD10 is expressed early during apoptosis but before DNA fragmentation, lymphocytes of a B-CLL case were exposed for 24 hrs to etoposide in vitro. Based on correlated expression of CD10 and PI, three different regions were recognized: the non apoptotic CD10/sub-G0 peak, the apoptosis-prone CD10/sub-G0 peak and the apoptotic CD10/sub-G0 peak* cell sub-populations. Furthermore, the same B-CLL cells were physically separated on the basis of their CD10 expression. As expected, CD10+ cells also showed a high proportion of Annexin V together with a significant amount of fragmented DNA. These findings indicate that CD10 expression may represent a valuable marker for apoptosis in B-CLL cells and suggest possible clinical applications in the evaluation of pharmacological efficacy in vivo.

PO232
PERIPHERAL BLOOD CD38 EXPRESSION PREDICTS TIME TO RE-TREATMENT IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS RESPONDING TO FIRST-LINE THERAPY WITH HIGH-DOSE CHLORAMBUCIL

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The aim of this study was to evaluate the prognostic impact of CD38 expression by peripheral blood lymphocytes on time to retreatment (TTR) in previously untreated B-CLL patients responding to first-line treatment with HD-CLB. Patients were prognostically stratified according to NCI-revised Rai and Binet stages, total tumor mass (TTM) score and bone marrow histology pattern. Out of 161 untreated cases evaluated for CD38 expression, 62 were subsequently treated with HD-CLB. Six patients failed to respond, 3 were unavailable for analysis, while 53 achieved complete (CR) and 20 partial response (PR). Low-dose chlorambucil as maintenance chemotherapy was given to 43 patients. The overall median TTR was 32.5 months (95% C.I., 25.5-39.5). Age at the time of treatment, sex, TTM score and bone marrow histology pattern failed to be significant predictors of TTR. Indeed, univariate analysis demonstrated that i) early Binet and NCI-modified Rai stages, ii) CR achievement iii) maintenance treatment with low-dose CLB and iv) CD38 expression below the median value for both percentage and mean fluorescence intensity (MFI) were associated with a statistically longer TTR. In multivariate analysis, the use of maintenance chemotherapy, the achievement of a CR status after HD-CLB and CD38 expression below the median values yielded an independent prognostic impact on TTR.
untreated chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma in leukemic phase (SLL). We treated 10 patients (6 males and 4 females; mean age 78, range 71-89). Treatment was based on the presence of signs and/or symptoms attributed to CLL (6 pts) or SLL (4 pts). Performance status (PS) before treatment was: 0 10% (1/10), 1 30% (3/10), 2 50% (5/10) and 3 10% (1/10). Treatment consisted of 6 weekly infusions of R at 375 mg/mg/week. Weekly dose was divided in two consecutive days until circulating lymphocytes were < 40,000/m3. In an intent-to-treat analysis the overall response rate was 90% (9/10). Four pts (40%) achieved a complete response (CR), 5 (50%) a partial response (PR) and 1 (10%) a stable disease (SD). Responses were distributed: CLL 100% (3 CR, 3 PR); SLL 75% (1 CR, 2 PR, 1 SD). All patients completed the therapy. Treatment was very well tolerated with an infusion-related syndrome noted only in 1 patient (hypotension, nausea and chills); infusion-related side effects resolved rapidly. The median time to progression has not yet been reached. One year progression-free survival is 60%. Duration of response remains to be determined. PS after treatment was: 0 30% (3/10), 1 60% (6/10), and 2 10% (1/10). Mean blood counts before versus after treatment in CLL were: circulating lymphocytes 66,700 vs 2,230/m3, neutrophils 1,880 vs 2,890/m3, Hb 10.8 vs 11.5g% mL, platelets 158,000 vs 153,700/m3. Mean bone marrow lymphocytes in SLL were 59% before treatment. CTX (3.5 gr/m2/die) and VP16 (300 mg/m2/die) for 2 days followed by daily rG-CSF (5 mg/kg s.c.). Leukapheresis collection of PCB was performed on the 12th day using Fresenius separator. The apheresis product contained 3.6x10^11 kg CD34+, and 29.7% of the total cells were CD19+ CD5+ (residual B-CLL cells). We performed conventional cryopreservation of thisuffy coat. After a month the patient was undergone a BEAM conditioning chemotherapy followed by reinfusion of the Mabthera manipulated autograft. In brief: PCB were rapidly thawed at 37°C and normal saline was infused. Non adverse reaction was shown during the infusion. Hematological recovery was on 16th day (PMN > 500 /m3) and on 24th day (Plts. > 50,000 /m3); complications were limited to neutropenic fever and mild mucositis. The patient is now in clinical hematological remission 26 months after transplant. This is a new concept of B-cell purging in B-CLL patients and it is our opinion that this method can be extended to all B-cell CD20+ lymphomas.

P0236 FLUDARABINE IN COMBINATION WITH CYTOXAN IS HIGHLY EFFECTIVE AS FRONT-LINE THERAPY IN ACTIVE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA


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Fludarabine (FLU) alone or in combination has been reported to be effective both in pretreated and untreated in B-CLL. Granulocytopenia and infection were the major adverse events. In vitro studies showed a synergic activity of FLU with other drugs, in particular with mitoxantrone, epirubicin and cyclophosphamide (Cy). In a retrospective study of 65 pretreated patients with B-CLL we obtained an high response rate (73%). Here we report the results of a retrospective study in a cohort of 15 untreated patients with B-CLL performed in 2 Departments of Hematology. All patients had a confirmed diagnosis of active B-CLL, were free of active bacterial or viral infection and were 18 years old or above. All patients were treated with FLU 30 mg/m2 days 1-3, i.v. plus Cy 300 mg/m2 days 1-5, i.v. plus Cy 300 mg/m2 days 1-3, i.v. every 4 weeks for at least 2 courses or till the best response. Patients received antibiotic prophylaxis throughout treatment and growth factor (G-CSF) when indicated. Hematological remission was achieved in 12/15 patients (80%) and 5 patients (33%) entered a complete remission with an improvement of their symptoms. In 2 patients progression occurred. The only serious adverse event was severe granulocytopenia with infections (1 case of pneumonia and 1 case of subdural empyema).
risk; median lymphocytes in peripheral blood were 40.3×10^9/l (range 5.2-100.0). All 15 patients received at least 2 courses (range 2-6, mean 4) and 14 were evaluated for toxicity and response. Only 4 patients experienced major hematomatological toxicity (WHO=3). We observed 6 CR (42.8%) of which were molecular CR and 6 PR (42.8%) Overall response was 85.6%. Refractory patients were 2 (14.3%). M can follow up is 9 months (range 1-31) and the mean response duration is 7 months (1-25). Two patients in PR showed DP and 1 is dead. Our preliminary data confirms the high efficacy of the Flu + CY regimen in previously untreated patients with B-CLL. Tolerability is good but these data need to be confirmed in a larger group of patients.

PO237
CLINICAL PROGNOSTIC FACTORS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA IN RAI STAGE 0: RETROSPECTIVE ANALYSIS OF 165 CASES

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From 1979 to 1999, 165 B-CLL patients in Rai stage 0, consecutively observed in our institution, 83 males, mean age of 66.6 years, were retrospectively evaluated. Survival: after a median follow-up of 57.7 months, 41 patients (24.8%) have died, 22 (13.3%) of CLL-related causes (disease progression, infections, Richter syndrome), with a median overall survival of 11.5 years, shorter than that of sex- and age-matched inhabitants of our region. Considering disease-related deaths, survival was similar to patients who died of non-CLL-related causes and to normals. No difference in specific survival was observed between smoldering or indolent CLL, according to M-ontserrat and the French group, cases and the remaining ones. Finally, no progressive patients showed the same survival as the normal population. In multivariate analysis, age over 60 years and disease progression were the two independent negative factors. Disease progression: 62/165 patients progressed in clinical stage or reached a Total tumor mass (TTM) score > 9, threshold for treatment in our institution. The median time to progression was 36.3 months. French criteria for smoldering disease, in both versions (A’ and A”), well identified patients with rapid progression. In multivariate analysis, four independent variables were identified, in order of relative risk (RR): i) lymphocyte doubling time (LDT) ≤ 12 months during the first year of observation (RR 16.0); ii) TTM > 5, corresponding to > 25×10^9/L (RR 4.6); iii) age > 60 years (RR 2.3); iv) peripheral lymphocytosis increase > 25% after the first year of observation (RR 2.0). Thus patients were divided into three groups with: i) no risk factor, 2) at least one with the exclusion of LDT< 12 months, 3) at least LDT<12 months, with progression free survival rate at 10 years of 63.2%±12.1%, 37.2%±7.7% and 0%, respectively (p=0.0001).
Study supported by AIL, Associazione Italiana contro le Leucemie, Sezione “Alberto Neri”, Reggio Calabria.

PO238
PERIPHERAL CD34+/CD19+/CD5- CELLS IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is widely considered a clonal expansion of neoplastic B-lymphocytes frozen at an intermediate stage of B-cell differentiation. Sporadic evidences, however, has been reported of abnormalities in the stem cell compartment of CLL patients, suggesting an origin of the leukemic process at a stage of B-cell maturation pathway earlier than that generally accepted. Since autologous stem cell transplantation represents a new therapeutic approach for selected subsets of CLL patients, the question of whether or not CD34+ cells could be intrinsically abnormal in B-CLL deserves further studies. In the present report, circulating CD34+ cells from patients with typical B-CLL have been studied by multiparametric flow cytometry in attempt to provide information on whether they could express the peculiar CD19+/CD5- B-CLL phenotypic pattern. Peripheral blood samples from 14 patients of both sexes with diagnostic CD5- B-CLL (7 untreated in early Rai stage, 7 treated in advanced Rai stage; mean age: 62.5 yrs) were evaluated. Seven healthy subjects, matched for sex and age, served as controls. Samples were first isolated through Ficoll-Hypaque, washed, resuspended and incubated with CD34 PE, CD5 FITC and CD19 PerCP conjugated antibodies. Multiparametric flow cytometric analysis, by combining FW-SC and RT-SC parameters with the three fluorescence signals, was performed with Cellquest software and at least 500,000 total events were acquired in list mode to a FACScalibur flow cytometer. Three color/isotypic controls and fluorescence background were always quantitated. All B-CLL patients showed detectable amount of circulating CD34+ cells, which were significantly higher than normal controls (mean 0.39±0.24% vs. 0.19±0.08%; p=0.01). No CD34+ cell co-expressing CD19+/CD5- could be detected in control subjects, whereas the majority of B-CLL patients (10/14; 71%) exhibited small but clearly detectable fractions (range: 0.02-0.2%) of peripheral CD34+ cells bearing CD19+/CD5- phenotype (p=0.01). No statistically significant correlation between these CD34+/CD19+/CD5- cells and either WBC (r=-0.1) or absolute lymphocyte count (r=-0.04) or Rai stage (r=-0.01) could be found. When we compared the untreated B-CLL patients with the treated ones we did not find any difference in CD34+ (p=0.58) and CD34+/CD19+/CD5- cell populations (p=0.75). These findings, although preliminary, provide evidence that a small subset of CD34+ cells co-expressing the typical B-CLL phenotype CD19+/CD5- circulate in peripheral blood of both untreated and treated CLL patients. Although this does not constitute demonstration of the leukemic lineage of the CD34+/CD19+/CD5- subset, it should recommend further investigations in this matter in order to guarantee the safety of therapeutic procedures such as autologous stem cell transplantation.
A highly variable clinical course characterizes B-cell chronic lymphocytic leukemia (B-CLL). In this view, the search for prognostic factors, such as the expression of surface molecules, in an attempt to discriminate which patients will experience a more aggressive disease, thus needing more intensive treatment, is a very exacting work for several investigators. We analyzed the clinical-biological features of 92 immunologically typical (CD5+ CD69+ CD23+) B-CLL patients stratified according to the expression of CD69, an antigen that was the earliest expressed on stimulated lymphocytes. Forty-eight (52%) patients expressed CD69 in more than 30% of CD19-positive cells, without differences by age, gender, typical or atypical morphology (FAB criteria), expression of surface membrane immunoglobulins between the two groups of patients. However, peripheral blood lymphocytosis (p = 0.001) and B and C Binet stages (p = 0.0007) were found to be closely associated with CD69 expression. In addition, in the CD69+ group t12 was more represented while del 13q14 was detected more frequently in the CD69- group (p = 0.03). Finally, median survival of CD69-positive B-CLL patients was 98 months, while it was not reached at 150 months in the CD69- patients (p = 0.006). In conclusion, our data clearly indicate that CD69 expression on neoplastic B-lymphocytes may be an additional useful tool to better recognize patients with an unfavorable clinical course in B-CLL.

PO240
EVIDENCE FOR ABNORMAL ANGIOGENESIS IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Recent reports have shown that angiogenesis is increased in B-cell chronic lymphocytic leukemia (B-CLL) patients. However, studies dealing with patients in early disease (i.e., Binet stage A) are absent. We addressed such an issue by measuring the degree of bone marrow (BM) angiogenesis in 45 Binet stage A patients and comparing it to normal control BM sections (n=12). The microvessels in BM sections were delineated by immunohistochemistry using antibodies to factor VIII. BM angiogenesis was evaluated as either microvessel area (mm² >10-²) or microvessel density (mean microvessel number per 600 × high power field [hpf]). The area occupied by microvessels was estimated by using the direct planimetric method of point counting as modified by Vacca et al. (1993). Microvessel area expressed as median value was increased in BM of stage A CLL patients (0.709 mm² x10-²; range, 0.451-1.428) in comparison to controls (0.090 mm² x10-²; range, 0.060-0.1260; p<0.0001, Mann-Whitney test). The median microvessel count/hpf was also higher in CLL patients (5.0) than in normals (0; p<0.0001). As a matter of fact, the two methods for assessing BM angiogenesis closely correlated to each other (r=0.958; p<0.0001). Increased BM angiogenesis did not reflect clinico-hematologic features of CLL such as the histologic pattern of BM involvement (p=0.5), absolute peripheral blood lymphocytosis (p=0.5), LDH (p=0.45), lymphocyte doubling time (p=0.5), β2-microglobulin (0.605). In the same cohort of patients we measured serum levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). When looking for association between serum concentration of these angiogenic cytokines and BM angiogenesis, a significant correlation was found between microvessel area and serum concentration of VEGF (p=0.01). Finally, the pattern of BM angiogenesis was tested as predictor of disease-progression. Stage A patients whose BM microvessel area was higher than the 75th percentile (0.9 mm² x10-²) were more likely to progress to a more advanced clinical stage than patients with BM microvessel area lower than 75th percentile (p=0.03). In conclusion, our results provide a clear evidence for an increased BM angiogenesis in CLL patients with early disease. Increased BM angiogenesis identify a subset of stage A patients at higher risk of disease-progression.

PO241
SERUM LEVELS OF INTERLEUKIN-6 CORRELATE WITH RISK OF DISEASE-PROGRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Interleukin-6 (IL-6) has relevant bioregulatory effects and serum levels of IL-6 are often increased in several lymphoid neoplasms. In B-cell chronic lymphocytic leukemia (CLL) constitutive cellular expression and serum release of IL-6 has been reported. More recently, a correlation between IL-6 serum levels and clinical outcome was reported in CLL (Blood 2001, 97:256). In an attempt to validate these observations, we measured IL-6 levels in frozen sera taken at the time of diagnosis in 60 CD5-positive B-CLL patients using a commercial ELISA assay. Levels of IL-6 ranged from 0 to 69.6 pg/mL (median, 0.80 pg/mL). Furthermore, they increased as a function of clinical stage (Stage A, median 0 pg/mL, range, 0-42.1 pg/mL; Stage B, median 5.4 pg/mL, range, 0-44.2 pg/mL; Stage C, median 6.3 pg/mL, range, 0-69.6 pg/mL; p = 0.001, Kruskal-Wallis test). The same applied when patients with different clinical stages were stratified into two groups according to median value of IL-6 (i.e., below and above 0.80 pg/mL; p=0.03). Increased levels of IL-6 did not parallel clinically-hematological parameters representative of tumor mass and/or disease-activity such as absolute peripheral blood lymphocytosis (p=0.485), histopathologic pattern of bone marrow involvement (p=0.915), lymphocyte doubling time (LDT) (p=0.686), β2-microglobulin (p=0.824). After a median follow-up time of 13 months (range, 2 to 40 months) 13 out of 41 (31.7%) Stage A patients progressed to a more advanced clinical stage (i.e., 6 to B and 7 to C), the risk of disease-progression at 24 months being 42.9%. Interestingly, patients whose IL-6 serum...
PO242 FLUDARABINE AND CYCLOPHOSPHAMIDE FOR THE TREATMENT OF ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA

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In vitro studies have shown synergistic activity of the combination of fludarabine (FLU) and cyclophosphamide (CY). In addition, recent investigations reported encouraging results of FLU-CY in patients with chronic lymphoproliferative disorders. The aim of our study was to assess the efficacy, toxicity and costs of FLU-CY combination in a series of 18 consecutive patients with chronic lymphocytic leukemia (CLL). From January 1999 to December 2000, 18 patients with advanced CLL were treated with FLU at 30 mg/m² and CY at 300 mg/m² daily for 3 days, intravenously. The median age was 62 years (range 46-74). Five patients (28%) received FLU-CY at the diagnosis (2 in Rai stage 4 and 3 in Rai stage 2 with bulky disease), while 13 (72%) had been previously treated (median chemotherapies: 1.5, range 1-5). In particular, 4 patients with CLL had been pretreated with FLU alone and 2 had relapsed from autologous stem cell transplantation (ASCT). Criteria for complete remission (CR) included disappearance of all palpable disease, normalization of blood count and no detectable CD5/CD20 positive cells at flow cytometry. Partial response (PR) was defined as 50% or more reduction of palpable disease as well as 50% improvement over baseline of hematologic parameters. Any other response was considered as a failure. Evaluation for response was done after 4 courses, at the end of treatment and every three months during the follow-up. 14 out of 18 (78%) patients achieved CR, 3/18 (17%) obtained PR and one (5%) showed progression of the disease. CR rate was 100% (5/5) for patients receiving FLU-CY as first line therapy and 69% (9/13) for pretreated patients. The main complication of therapy was related to severe neutropenia (granulocytes less than 500×10⁹/L), which occurred in 7 out of 18 patients (39%) and required G-CSF administration. Pneumonia or sepsis occurred in 6 cases (33%), resulting in 1 death in the patient relapsed from ASCT, while additional 5 patients experienced FUO. Blood transfusions were required in 4 patients (22%). The median CR duration was 10 months (range 1-14). Ten patients are alive in continuous CR at the time of writing. Our data demonstrate the feasibility and efficacy of FLU-CY combination regimen in a patient population suffering from CLL, with a median age of 62 yrs. In addition, as compared to the standard FLU treatment (25 mg/m² for 5 days), a reduction of costs of more than 40% was registered (cost of 6 cycles 12800 € for FLU vs. 7720 € for FLU-CY, respectively), FLU-CY requiring three days of hospital admission and reduction of 28% FLU amount. In conclusion, although myelosuppression and infections remain major complications of FLU-CY, raising the opportuneness of prophylactic use of G-CSF in the elderly patient population at least, the high efficacy and the reduced costs of such a therapeutic regimen, make it a candidate for first line treatment of advanced CLL.

PO243 INCIDENCE OF SECOND MALIGNANCY IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: RETROSPECTIVE STUDY ON 642 PATIENTS FROM A SINGLE INSTITUTION

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The incidence of second malignancy (SM) was retrospectively analyzed on 642 chronic lymphocytic leukemia (CLL) patients consecutively observed in our institution. SM was found in 89 patients (13.8%): in 13 of them before CLL diagnosis, in 8 at the same time and in the remaining 68 subsequently (51.4% within the first five years). Skin (19.1%), gastrointestinal tract (17.9%), lung (17.9%), breast (11.2%) and prostate (5.6%) were the most frequently involved sites. Transformation into aggressive non-Hodgkin’s Lymphoma (Richter syndrome) occurred in 17.9% of cases. After excluding cases with SM preceding CLL, 629 patients were accounted, 352 males and 277 females, mean age 66.2±10.3 years and with the following stage distribution at diagnosis: 408 in stage A according to Binet, 118 B, 59 C; 216 stage O according to Rai, 304 I-II, 65 III-IV. At the time of analysis, 224 patients are alive, 226 dead and 178 lost to observation. Death causes were: 41.1% CLL progression, 17.6% infection, 9.2% SM, 3.0% Richter syndrome, 28.6% other causes. In univariate analysis, only stage according to Binet showed a nearly significant relationship (p=0.051) with SM occurrence; in fact, 70.8% of SM were diagnosed in stage A cases, as expected because of their longer survival. In order to investigate the impact of chemotherapy on SM incidence, also patients with SM contemporary to CLL diagnosis were excluded; on the remaining 621 cases, 68 second malignancies were reported. A trend for a higher SM frequency in treated patients, 50/68 (73%), was found as compared with untreated ones (p=0.139). Both treatment and Binet stage entered a multivariate analysis model and both resulted significant with a relative risk of SM occurrence of 2.2 and 1.1, respectively.

Study supported by AIL, Associazione Italiana contro le Leucemie, Sezione “Alberto Neri”, Reggio Calabria.

PO244 SECOND AND SUBSEQUENT MALIGNANCIES IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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We have analyzed second malignancies (SM) and family malignancies in a group of 347 pts affected by chronic lymphocytic leukemia. SM were present in 62 patients (17.9%): in 16 diagnosis was contemporaneous with the diagnosis of CLL; in 14...
patients a SM was diagnosed within two years from the diagnosis of CLL, in 15 patients between 2 and 8 years, while in 9 patients it was diagnosed 8 years or more after CLL. Skin (26%), lung (17%) and gastrointestinal tract (14%), breast (8%), prostate (6%), kidney (6%) were the most frequent sites of SM. In 29/62 cases SM developed in patients who had never received antileukemic therapy: this underlines the importance of the immune defects associated with CLL in the genesis of this severe complication. A family survey has shown that 19/347 (5.5%) patients had at least one first-degree relative affected by a hematological neoplasm. Overall 23 cases were found: five CLL, nine acute lymphoblastic leukemias, one acute myeloblastic leukemia, eight non-Hodgkin's lymphomas. Three families with more than one individual affected by CLL were identified. Family #1: two brothers, aged 63 and 61 at diagnosis, were affected by stage A CLL according to Binet: they are still out of therapy with no signs of progression after 6 and 5 years from diagnosis. Family #2: three brothers: one patient with stage A at the onset progressed and received treatment after 19 months; one died of lung cancer. no follow-up data are available about the third brother. Family #3 is composed by three sisters; one of them is out of therapy after 10 months after diagnosis; no follow-up data are available about the other two sisters.

P0245
RITUXIMAB AS SINGLE AGENT IN LYMPHOPROLIFERATIVE DISORDERS REFRACTORY TO CHEMOTHERAPY: PRELIMINARY RESULTS

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Rituximab (Mabthera) is a humanized murine anti-CD20 monoclonal antibody used in the treatment of follicular and low grade lymphomas. In accordance with literature showing good safety and tolerability of the molecule, we decided to treat patients with refractory lymphoproliferative disorders. The schedule utilized was 375 mg/m² in weekly administrations. We have treated 6 CLL and 2 low grade NHL; all patients were refractory to the first administration, were correlated with leukocytosis with severe thrombocytopenia, platelet count showed an average reduction of 50,000/m3. The two patients with NHL did not show any important change of lymph node diameters. During the treatment, hematological toxicity and infectious complications were not observed. The remission time was from 5 to 8 months. In two relapsed CLL patients, we have repeated a second course of therapy, obtaining the same efficacy and remission duration. In a patient with NHL a maintenance treatment with monthly administration was tried, but the results were unsatisfactory. Our experience, although limited to a few cases, shows that rituximab therapy is an effective and well tolerated treatment in CLL patients with resistant leukocytosis; it seems less effective in monotherapy in refractory low grade NHL patients.

P0246
PROGNOSTIC SIGNIFICANCE OF CD38 AND FMC7 EXPRESSION IN B-CHRONIC LYMPHOCYTIC LEUKEMIA

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The clinical course of B-CLL is variable. Whereas some patients with CLL have a normal life span, surviving prolonged periods without requiring therapy, others die within five years after diagnosis despite aggressive treatment. Recent data indicate that CD38 expression may be associated with unmutated IgV genes and that CD38 positive cases are characterized by a poor outcome. Furthermore the expression of FM C7 was correlated to trisomy 12, atypical morphology and worse prognosis. The aim of our study was to investigate whether CD38 and FMC7 expression may predict the clinical outcome of B-CLL patients. To assess the prognostic significance of CD38 and FM C7 expression in B-CLL, we analyzed the relationships among these phenotypes and clinical presenting features, treatment history and survival in 167 patients (97 male/70 female, median age 62 years) consecutively admitted at our Institute from January 1993 to December 1999. All cases were classified as B-CLL according to the staging system proposed by Matuses et al. (1994). With regard to CD38 or FMC7 expression, the threshold of positivity was set at 30%. Patients were also classified according to the percentage of B-CLL cells expressing CD38 or FM C7 into ≥30% and <30% groups. CLL was defined as active or progressive according to the NCI revised guidelines for diagnosis and treatment (1996). With regard to CD38 or FM C7 expression, 32 out of 162 evaluable cases (19%) were positive for CD38, and 58 out of 165 evaluable cases (35%) were positive for FM C7. The CD38 positivity was associated with a higher incidence of intermediate/high modified Rai stage (46% vs 38%) and Binet’s stage B or C (30% vs 20%), active or progressive disease (31% vs 17%) and a worse overall survival (median: 98 vs 126 months, p=0.07). FM C7 positivity was associated with a lower incidence of intermediate/high modified Rai stage (38% vs 42%) and Binet’s stage B or C (18% vs 26%), active or progressive disease (17% vs 20%) and a better overall survival (median: n.r. vs 120 months, p=n.s.). Of interest, the analysis of the survival of stage A patients and of the progression-free survival according to CD38 and FM C7 expression did not show any significant difference. We conclude that immunophenotypic analysis of CD38 and FM C7 expression has limited prognostic significance.
PO247
FLOW CYTOMETRIC IMMUNOPHENOTYPING EFFECTIVENESS IN DISCRIMINATING CHRONIC LYMPHOID LEUKEMIA FROM OTHER LYMPHOPROLIFERATIVE DISORDERS

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The role of flow cytometric immunophenotyping (FCI) in chronic lymphoid neoplasms has become crucial in recent years, because of the availability of new monoclonal antibodies (MoAbs) which dramatically improved the diagnostic reliability of FCI as long as the definition of each pathological entity recognized by REAL classification. In our institution, we evaluated by FCI, from January 2000 until April 2001, 27 cases of chronic lymphoid leukemia (B-CLL) and 14 cases of B non Hodgkin’s lymphoma (B-NHL). We used the following MoAbs combination: CD5/CD19/CD45, CD20/CD23/CD45, CD20/CD25/CD45, FM C7/CD22/CD45, CD19/CD11c/CD45, CD19/CD79b/CD45, CD19/CD38/CD45, sIg/CD19, sk/sIg/CD19. Data acquisition and analysis were performed with a FACScalibur flow cytometer and the CellQuest software (Becton Dickinson), using a CD45- or CD19-based gating strategy on lymphoid population. The positivity for a given MoAb was attributed when expressed by at least 30% of the B-lymphoid cells. The results were the following: as expected, CD20 was positive in 100% of both CLL and NHL cases; CD5 was positive in 100% of CLL and in 71% of NHL patients; noteworthy, we never found CD5-negative CLL; very similarly, CD23 was positive in 100% of CLL against 21% of NHL cases. No major disparity was found about CD25 expression, which was respectively positive in 72% and 62% of CLL and NHL cases; likewise, CD11c was positive in 62% and 57% of CLL and NHL cases; when mean fluorescence intensity (MFI) was considered, no significant difference was found in either CD25 or CD11c expression in the two groups (p=0.575 and p=0.411, respectively). sIg expression resulted rarely weak in the CLL in comparison to the NHL group (15% vs. 90%). More interestingly, CD222 resulted always positive both in CLL and NHL cases, but when comparing MFI mean values in the two groups (50.61 and 121.17), we found a significant difference (p<0.05) with typically weak expression in the CLL group; CD79b was even more informative: 47% of CLL cases were positive against 100% of NHL cases with very different MFI mean values: 14.68 vs. 65.21 (p<0.05). Nevertheless, in our experience, CD79b resulted more frequently expressed in CLL than reported in literature (about 5% of CLL). FM C7 antigen, considered generally negative in CLL, resulted positive in 42% of CLL vs. 71% of NHL cases, with generally lower MFI mean values in CLL: 16.34 vs. 44.77 (p<0.01). Again, FM C7 positivity resulted, in our CLL series, more frequent than expected. Finally, CD38, whose prognostic significance appears important in CLL according to some reports, resulted expressed in 23.5% of CLL cases, against 90% of NHL cases. In conclusion, we confirm the high diagnostic value of CD5, CD23, CD22dim and weak sIg in discriminating CLL from other lymphoproliferative disorders, and, in contrast, the poor relevance of CD25 and CD11c expression. On the other hand, our observations suggest that FM C7 expression is more variable, but generally with lower fluorescence intensity in CLL; similarly, CD79b is more often expressed in CLL than reported in other series. However, these discrepancies need to be confirmed in larger series.

PO248
PROGNOSTIC SIGNIFICANCE OF PERIPHERAL BLOOD LYMPHOCYTE MORPHOLOGY IN PATIENTS WITH ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH FLUDARABINE + PREDNISONE

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In 69 patients with advanced chronic lymphocytic leukemia (CLL) (CD5+, CD20+, CD23+), the prognostic significance of peripheral blood lymphocyte morphology before the start of first line fludarabine + prednisone (FD + P) therapy was retrospectively analyzed on stored film preparations. Three groups of patients according to the peripheral blood lymphocyte morphology evaluated prior to FD + P therapy were identified. The larger group of 42 patients showed a typical CLL morphology (T) characterized by small mature lymphocytes and <11% of atypical lymphocytes. Twenty-seven patients formed a group with >11% of lymphocytes with atypical morphology; a morphologic pattern of CLL/PLL, with prolymphocyte prevalence, was observed in 13 of the above patients (P), while a cleaved lymphocyte prevalence was observed in 14 patients (C). All patients were treated with FD (25 mg/m2 × 5 consecutive days every 4 weeks) associated with P (40 mg/m2 × 5 consecutive days every 4 weeks). Response was assessed according to National Cancer Institute criteria. No statistical differences in the three groups (T, P, C) were observed in the distribution of clinical features prior to therapy (median age, gender, stage, lymphocyte count, LDT, BM histology, CLL duration). The morphologic pattern before FD+P therapy did not influence statistically the response rate to therapy and the duration of response. Patients with atypical lymphocyte (P+C) morphology were characterized by a significantly shorter survival duration than patients with typical morphology (T) (p<0.05). However, in multivariate analysis the lymphocyte morphology before FD+P therapy lost its significance, while two parameters, age (€55 vs >55 years) and CLL duration prior to FD+P therapy (€12 vs >12 months), emerged as significant and independent prognostic factors on survival probability.

PO249
CD38 AND PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA: PRELIMINARY RESULTS

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The search for prognostic markers in CLL has always been a subject of hematological studies; the various parameters proposed (iJ2-microglobulin, soluble CD23, thymidine kinase, bone marrow histology, cytogentic anomalies, etc.) are only partially correlated with clinical data; in addition, for some of them the method is complex and scarcely reproducible. CD38 is one of the latest prognostic markers proposed in literature; this antigen seems to be strictly correlated with the phenomenon of Ig gene somatic hypermutation. CD38 divides CLL into two diseases,
PO250
IMMUNOPHENOTYPIC ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTES IN PROGRESSED B-CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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B-cell chronic lymphocytic leukemia (B-CLL) is a neoplastic disorder characterized by proliferation and accumulation of monoclonal long-lived and immunoincompetent B lymphocytes in the peripheral blood, bone marrow and lymphoid tissues. Diagnostic criteria are based on morphological aspects of the leukemic B-cells and on their antigenic pattern. The aim of this study was to investigate whether clinical and/or cytomorphological progression observed in B-CLL patients, does not correlate with any change in the antigenic expression. For this purpose, 16 B-CLL patients, progressed 4-14 years from the onset of the disease, entered the study. Immunophenotypic analysis was carried out on peripheral blood lymphocytes in flow cytometry (FACS Scan, Becton Dickinson) using direct immunofluorescence technique by dual color staining employing a panel of conjugates (FACScan, Becton Dickinson) using direct immunofluorescence

PO251
SERUM LEVELS OF SOLUBLE CD25 IN MONITORING THE OUTCOME AFTER PURINE ANALOGS IN HAIRY CELL LEUKEMIA

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Purine analogs (PA), cladribine (CDA) and deoxycoformycin (DCF) are considered presently the most active treatment for hairy cell leukemia (HCL), producing high rates of complete remission (CR) (70-80%), even in patients relapsed or resistant after α-interferon (IFN). Soluble CD25 (sCD25) serum level represents a reliable marker of disease bulk in HCL, and its measurement is a simple and suitable method for monitoring the response to therapy. We measured sCD25 serum levels in 32 patients with HCL receiving PA; 20 relapsed after IFN and was treated with CDA (9 patients) or DCF (11 patients), and 12 were given DCF as first line treatment. After PA, 16 patients achieved CR, 7 minimal residual disease (MRD) detectable only by immunostaic methods (CR+MRD =72%), 8 partial remission (PR) and 1 did not respond (NR). Of the 20 patients previously treated with IFN none had achieved CR (3 MRD, 15 PR, 2 NR). Mean sCD25 (U/mL±SD) serum levels, highly increased before treatment (17,458±14,659), progressively decreased after PA to a minimum at the 6th month (655±457) significantly lower than values detected after IFN (2,589±734). Mean sCD25 values after PA became significantly lower in patients achieving CR/MRD (478±220) than in patients with PR/NR (1,162±596). In particular, sCD25 became normal (i.e. <500U/mL) in all but 3 patients achieving CR or MRD. In patients with PR or otherwise classifiable as CR but with spleen enlargement detectable only by ultrasound (2 cases), sCD25 remained above normal level. So far, 8 relapses have occurred, 4 after PR and 4 after CR, or MRD. In 4 patients, relapsed after CR/MRD, sCD25 increased levels (688 to 1,702 U/mL) preceded the evidence of relapse by 12 to 30 months. In conclusion, the higher response rate obtained in HCL with purine analogs, compared to IFN, is paralleled by lower sCD25 levels. sCD25 is also useful in monitoring subsequent follow-up, heralding the relapse and reducing the need for bone marrow biopsies.
phamide 60 mg/m²/day was added to therapy. After an initial mild treatment (prednisone 1 mg/kg/day p.o); after 45 days, cyclophosphamide was prescribed every 15 days. After 3 administrations, the Hb level rose to 9.0 g/dL, therefore therapy was continued for 5 months. Subsequently the treatment was delayed monthly and stopped in four months. No transfusions was required during therapy. At the end of therapy, HGB level rose to 10.3 g/dL and DAT was negative. Interestingly, during the post-therapy period Hb level progressively increased to 11.6 g/dL. No AIHA relapse occurred. After 74 months that CLL was diagnosed, the patient died of cardiac disease. AIHA is a frequent complication of CLL as well as the related therapy such as chlorambucil and purine analogues. In particular, it seems that therapy with fludarabine in heavily pretreated patients leads to an alteration of CD4-CD8 ratio leading auto reactive clones. For this reason, our patient was given very low doses of fludarabine. The patient was carefully monitored during the entire period in order to suspend therapy in case clinical profile deteriorates. This report suggests that remission could be achieved with Fludarabine in patients with resistant warm agglutinin-induced haemolysis. The rationale for this treatment’s schedule is founded, firstly on the possibility that low-doses of Fludarabine are efficacious in untreated patients; secondly on the conviction that a CLL-related cause exists in the rising of AIHA. Most likely, the relationship between CLL, AIHA, efficacy and toxicity of fludarabine therapy is complex. Further studies are needed in order to clarify this biological process.

PO254 CONCOMITANT EVIDENCE OF CHRONIC LYMPHOCYTIC LEUKEMIA AND MULTIPLE MYELOMA IN THE SAME PATIENT

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CLL is frequently complicated by the development of second malignancies, besides it is known that about 5% of CLL transform to aggressive lymphoma (Richter syndrome); however, the coexistence of two clonal lymphoid pathologies in the same patient is a rare event. Case report. A 61-year old man first seen at our hospital because of severe hypotension and hypoalgesia of the legs associate to back pain. A neurological examination diagnosed a Brown-Séquard syndrome. NMR of the spinal column revealed an expansive process at DT4 level with medullary compression. Surgical intervention of medullary decompression and laminectomy on DT4 was performed with gradual regression of the symptoms. The istiological biopsy specimen of the mass was consistent with plasmocytoma with kappa chains expression; instead the bony fragments presented a interstitial lymphoid infiltration immunophenotypically consistent with CLL (CD19+CD20+ CD5+CD23+ low intensity sIg). The blood count demonstrated leucocytosis (WBC 46,000/µL) with lymphocytosis and thrombocytopenia (Plt=70,000/µL), additionally a serum protein electrophoresis showed a monoclonal component IgGκ of 0.7 g/dL. Total body scan demonstrated splenomegaly and small lymphonodes: laterocervical, axilla, lomboaortic and para-caval. Bone marrow aspirate showed a lymphoid infiltration of around 80%, plasmacellular component was normal. Bone marrow immunophenotype was consistent with CLL (CD19+CD20+ CD5+CD23+ low intensity sIg). Diagnosis of CLL Rai stage IV was made. The patient received local radiotherapy at D4 level followed by legs mobility and sensibility recovery. Besides he underwent chemotherapy with Chlorambucil 16 mg/m² every 3 weeks for 15 total courses. Twelve months later a progressive increase of the monoclonal component up to 4.2 g/dL was observed; a new bone marrow biopsy showed a plasmacellular infiltration of 40%, BrdU 4%. Because of this feature of multiple myeloma the patient was treated with melphalan+prednisone and VCAP (vincristine, adriamycin, cyclophosphamide, prednisone) without any response. Therefore we administered a salvage treatment D-CEP (cisplatin, cyclophosphamide, etoposide, prednisone) following peripheral blood stem cell collection in the prospect of autotransplantation.
PO255
SEEDING OF HEMOPOIETIC PROGENITOR CELL OF KAPOSI’S SARCOMA WITH SOLID ORGAN TRANSPLANTATION


The long term survival of donor lymphoid cells in the recipients of solid-organ transplants has been well established. The development of acute promyelocytic leukemia in a liver transplant female patient, two years after transplantation, bearing the genetic and phenotypic markers of the male donor, has also indicated the possible transmission, survival and leukemic transformation of donor myeloid progenitor cells in solid organ recipients (Bodo et al. N Engl J Med 1999; 341:3407). However, the fate of other hemoepoietic progenitors in organ allografts is yet to be determined. Kaposi’s sarcoma (KS) is a vascular tumor characterized by the proliferation of spindle cells which may originate from a circulating peripheral blood hemoepoietic precursor cell with a spindle-like shape and expressing endothelial and macrophage antigens (Browning et al. Blood 1994; 84:2711). KS associated herpesvirus or human herpesvirus 8 (KSHV or HHV-8) has been recognized to infect the neoplastic spindle cells and to be necessary for the development of this tumor. We used immunohistochemical and molecular methods to test the hypothesis that the cutaneous KS which has developed, simultaneously, in 2 renal transplant female patients, 20 months after receiving twin kidneys from the same cadaver male donor (Luppi et al. Blood 2000; 96:3279), was of donor origin. We applied immunohistochemical analysis for a latent specific antigen (LNA1/orf73) of HHV-8 in association with polymerase chain reaction (PCR) analysis of single micromanipulated cells for Y chromosome and HLA genes of the male donor as well as for a specific genomic sequence (orf-26) of HHV-8 on the posttransplant KS lesions of the 2 renal transplant female patients. Single cells were isolated by micromanipulation from LNA/orf73-stained tissue sections of cutaneous KS lesions of both patients. The spindle and endothelial cells were identified and isolated as LNA1/orf73-positive, and used in the PCR experiments. From each of 2 cases studied, between 10 and 25 individual spindle and endothelial cells were analyzed. The amplification efficiencies for chromosome Y, HLA gene (DR4) and HHV-8 (orf26) ranged from 7% to 15%. These values were in the same range as those obtained in previous studies and due to technical matters such as degradation or inaccessibility of the DNA and missing of part of the nucleous for many micromanipulated cells. We detected the presence of genetic markers of the donor (chromosome Y and HLA DR4 gene) in the spindle and endothelial HHV-8 infected cells localized in the cutaneous KS lesions from the female recipients, indicating, unequivocally, that the KS neoplastic cells in the 2 recipients were of donor origin. Our study shows, for the first time, that not only the KS associated herpesvirus but also the still elusive KS hemoepoietic progenitor cell, possibly infected with the virus, may be seeded with a solid organ transplant, survive in a recipient host and transform.

PO256
TREATMENT WITH BASILIXIMAB (SIMULECT) IN A CASE OF ACUTE GRAFT REJECTION AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION IN A PATIENT AFFECTED BY ACUTE NON LYMPHOID LEUKAEMIA


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Basiliximab is an IgG chimeric MoAb, specifically binding to and blocking the interleukin-2 receptor α-chain on the surface of activated T-lymphocytes. Up to now, basiliximab has been exclusively used for the prophylaxis of acute organ rejection, while no data are available in the field of graft rejection after BMT. We herein report a case of a 36-year old man affected by ANLL (FAB M4), in 2nd CR, relapsed after autologous PSCT. Lacking an HLA-identical donor, the patient underwent an HLA-haploidentical PBSCT from a sibling donor. Conditioning consisted of thiopeta, FAMP, ATG and melphalan. CD34 enrichment together with T and B lymphocyte depletion were carried out by Isolux 300i. The final number of CD34+, T+ and B+ cells were 8.7×10³/kg, 10×10³/kg and 13×10³/kg, respectively. An antibiotic resistant fever developed at day +5, continuing even after granulocyte engraftment (+11). At day +15, since granulocyte count slowed down, LDH level increased and the lymphomonocytoid CD8+ cells re-appeared, a diagnosis of graft rejection was made. Therefore, a new immunosuppressive treatment was initiated with CTX, FAMP and ATG followed by a second infusion of CD34-enriched cells from the same donor. Again, a new hematological picture of graft rejection was documented at day +11. Therefore, we administered 2 cycles of basiliximab (20 mg/each) in 4 days. A mixed chimerism was demonstrated at day +20. Lymphomonocytoid CD8+ cells disappeared together with fever at day +29 and a satisfactory hematopoietic recovery was noted. In conclusion, basiliximab could represent a new therapeutic option for the treatment of graft rejection after BMT.

PO257
CORD BLOOD AND MARROW UNRELATED DONOR SEARCH FOR HIGH RISK LEUKEMIC PATIENTS: RESULTS OF A PROSPECTIVE STUDY


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The search for an unrelated donor has been progressively increasing for patients candidate for an allogeneic hemoepoietic stem cell (HSC) transplant and lacking a suitable family donor. As umbilical cord blood (CB) has been proven to be a source of HSC, an increasing number of unrelated transplants has been performed using CB units provided by International CB
Banks as alternatives to International Bone Marrow Donor Registries. At present, tacking in account the alternative source of HSC (CB vs marrow unrelated donor [MUD]) and the start time of the search, no data are available on the therapeutic impact on survival of the unrelated transplant for the entire population of patients candidate for an allogeneic HSC transplantation. Between April 1995 and December 2000, 165 patients with high risk leukemia (ALL n=96; CML n=33; AML n=36), lacking an HLA identical family donor were considered eligible for an HSC transplant from an unrelated donor. Therefore, the search was simultaneously addressed to the Bone Marrow Donors Word-wide (BMDDW) through the Italian Bone Marrow Donor Registry (IBMDDR) and to the CB banks of New York and Netcord Organization. At a median time of 2 days (range 1-368) a median of 9 (1-66) suitable CB units was reported by banks for 145 (88%) candidates. The CB transplant eligibility was defined for 89 potential recipients after a median time of 44 days (1-363). According to our selection criteria, 31 patients underwent CB transplant at a median time of 106 days (49-321). On the other hand, the preliminary search for MUD recruited a total number of 76820 potential donors for 150 (91%) patients at a median time of 1 day (1-11). Because of the administrative procedures, the formal search started after a median time of 34 days (2-509) for 116 of 150 patients. Only 59 patients were still eligible for transplant when a definitive donor was found after a median time of 91 days (34-558) and until now 25 patients have been grafted at a median time of 210 days (120-614). By comparing CB vs MUD search the analysis of time to full eligibility showed a highly significant difference (44 vs 91 days; p=0.000). Consequently, patients received a CB transplant significantly earlier than recipients of a MUD transplant (106 vs 210 days; p=0.000). Overall survival for CB and MUD recipients from time of transplant was 43% and 34% respectively. Probability of survival calculated for the entire population (N=165) from the start of the search by censoring patients at transplant (Mantel-Byar method), shows a 10% advantage (24% vs 14%) but it does not reach a statistic significance (p=NS). In our study CB transplant represents a feasible and effective therapeutic option available for a higher number of patients with high risk leukemia.

PO259
SINGLE ALLOGENEIC STEM CELL TRANSPLANTATION AND DOUBLE AUTOLOGOUS-ALLOGENEIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA

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From April 1989 to October 2000, 70 MM patients received allogeneic stem cell transplants (allo SCT) at the Institute of Hematology and Medical Oncology “Seràgnoli”. Their median age was 43 years, 66% were in stage III, 57% had refractory or progressive disease. Preparative regimen consisted of Bu-Cy 4 in 27 patients and TBI+Cy+ MEL(120 mg/m²) in 43 patients. GVHD prophylaxis was accomplished using CsA+MTX in 74% of patients and T-cell depletion in the others. Stem cell source was bone marrow in 42 cases, while peripheral blood was used in 28 out of 33 patients transplanted from 1995 on. Among these latter patients TRM was 18%, a value significantly lower compared to that observed among patients transplanted in earlier periods of the study (38%). Stringently defined complete remission (CR) rate did not change over time; it was in the 37% range on an intent-to-treat basis and 44% among patients who could be evaluated. The 5-year projected probability of relapse averaged 55%, with no plateau in the relapse-free survival curve. Female sex and lower clinical stage before allo SCT were the only variables favorably affecting transplant outcome in a multivariate analysis. Between September 1994 and November 1998, 17 consecutive patients (65% stage III; 53% refractory to previous therapy) entered a pilot study of tandem (sub)myeloablative therapy with Mel (100 mg/m²) and autologous PBSC support followed by allo SCT. For comparison of their outcome, 17 pair mates were selected among patients not receiving Mel+ autologous PBSC support before allo SCT to match for sex, clinical stage and disease status. No significant difference between the two groups was observed in terms of TRM at 100 and 365 days. Although response status before allo SCT was upgraded in 56% of patients receiving MEL, their ultimate rate of CR following allotransplant was not different in comparison with the control group. Similar rates of relapse between the two groups were also observed at 3 years after allo SCT. It is concluded that 1) survival after allotransplant for MM has significantly improved from 1995 on, due to a lower TRM; 2) resistance to myeloablative therapy remains a major problem affecting the outcome of allo SCT, resulting in a low fraction of cures. Efforts to improve the results by the combined use of (sub/non) myeloablative therapy and immune-based strategies will be important avenues of investigational clinical trials in the near future.

PO259
THE ROLE OF MAGNETIC RESONANCE IMAGING IN THE PATIENT WHO PRESENTS NEUROLOGICAL SYMPTOMS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Following allogeneic human stem-cell transplantation (HSCT), 30-60% of patients develop neurological complications. The neurological symptoms are various including mild manifestations as well as more severe neurological symptoms like seizures, disturbances of consciousness and also coma. In many of these cases, differential diagnosis represents an important clinical problem that may considerably influence an early and effective treatment. Under this point of view magnetic resonance imaging (MRI) may have an important role because of its high sensitivity for the detection of toxic, inflammatory, ischemic, hemorrhagic and neoplastic disorders of the central nervous system (CNS). Brain MRI was performed in 20 patients who developed complex neurological symptoms after allogeneic HSCT for hematological malignancies. The MRI examination was positive in 85% of all cases. In the patients who presented negative MRI (15%) the PCR analysis of cerebrospinal fluid revealed the presence of human herpesvirus type 6 (HHV-6) in one case, while in the other two cases it was not possible to formulate a diagnosis. In the patients who had positive MRI the following radio-
logical diagnoses were formulated: 1) cyclosporine neurotoxicity (20%), 2) cerebrovascular disorders (5%), 3) corticosteroid-induced brain atrophy (10%), 4) CNS infection (25%), 5) CNS involvement for relapsing disease (15%) and 6) non-specific lesions (10%). The most common changes seen in cyclosporine-induced neurotoxicity are hyperintensive white matter lesions on T2-weighted MR images located typically at the level of the posterior regions of the brain. Brain atrophy has been described for other diseases with high doses of corticosteroids and is generally reversible after the discontinuation of corticosteroids. The MRI appearances in the patients suspected to have CNS infection are very heterogeneous. In one case of HHV-6 encephalitis, MRI showed diffuse, bilateral grey matter lesions with high signal intensity on T2-weighted images at the level of the temporal, parietal and occipital lobes. The lesions did not show any enhancement on post-contrast T1-weighted images. The diagnosis was confirmed by PCR analysis of cerebrospinal fluid. One case presented MRI suggestive of viral encephalitis characterised by subcortical white matter and cortical grey matter lesions of the temporal lobes. Some of the lesions showed high signal intensity on T1-weighted images suggestive for hemorrhages. All lesions showed only a minimal contrast-enhancement typically seen in the immunosuppressed patient due to a reduced inflammatory response. Two patients had MRI characterised by multiple focal lesions suspicious of septic emboli. The lesions showed high signal intensity on T2-weighted images and ring-enhancement on post-contrast T1-weighted images. In patients relapsed with CNS involvement MRI was characterized by parenchymal masses located at various levels, hyperintense on T2-weighted images and with marked enhancement on post-contrast T1-weighted images. Two patients showed periventricular white matter lesions, hyperintensive on T2-weighted images. The lesions were non-specific and could not be associated with any disease. In conclusion, in patients presenting neurological symptoms after HSCT brain MRI, notwithstanding it might not allow in any case a definitive etiologic diagnosis, shows features very suggestive of a specific pathology, that might be later confirmed by further analysis, especially in the case of CNS infections.

PO261
AUTOGRAPHING FOLLOWED BY NON MYELOABLATIVE ALLOGRAFTING FOR TREATMENT OF MULTIPLE MYELOMA
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Hematopoietic stem cell allografting is the only potential cure for multiple myeloma (MM). Unfortunately, MM patients tolerate myeloablative conditioning regimens very poorly with a high early transplant-related mortality (TRM). We conducted a pilot study using a two phase approach consisting of high dose melphalan (200 mg/m²) and autografting followed (40 to 120 days later) by a non-myeloablative allografting (NMA) with low dose total body irradiation (200 rads) and immunosuppression with cyclosporin (day-1+56) and micofenolate mofetil (day 0+27). The rationale of this protocol, designed by the Seattle group, is to separate temporally the high dose chemotherapy from allografting, in the attempt to combine the benefits of autografting (higher disease response and prolonged survival compared to conventional chemotherapy) and allografting (graft versus myeloma) reducing transplant-related toxicities. Eleven MM patients have been treated with this approach. Five, median age 51, (range 34-53) have completed the treatment phases and are evaluable at least 107 days post-NMA. All patients engrafted with a median of 8 days of neutropenia (ANC<500/µL) (range 0-11) and a median platelet count nadir of 80000/µL (range 64000-174000/µL). Only one patient required RBC transfusions prior to engraftment. Lymphopenia was noticed in all patients during the first 6 months post transplant, but normal CD4/CD8 ratio was observed at 2 months in all patients except one. Four out 5 showed CMV reactivation with positive antigenemia responsive to anti-viral therapy at a median of day +35 (range 24-68) post transplant. Grade II GVHD developed in 2/5 patients,
grade III-IV GVHD developed in 1/5. All patients had evidence of disease at the time of NMA. One patient reached molecular remission at 6 months, one achieved complete remission (CR) with disappearance of the monoclonal paraprotein by immunofixation at 3 months. Interestingly, these two patients developed only grade I GVHD not requiring therapy. The remaining three patients obtained a partial remission (PR). At a median follow up of 215 days (range 107-350), 4/5 patients are alive with Karnofsky score of 90-100%, two in CR, two showing a progressive gradual reduction of the disease. One patient, after achieving PR, died at 6 months post transplant of disseminated aspergillosis and grade IV GVHD. Our preliminary results are encouraging showing no early TRM and demonstrating that molecular remissions can be achieved with this two phase approach. Multi-center studies are under way to assess the value of this treatment on larger series of patients.

PO262
HYPERFRACTIONATED TOTAL BODY IRRADIATION AS CONDITIONING FOR ALLOGENIC STEM CELL TRANSPLANTATION IN 129 PATIENTS AFFECTED BY HEMATOLOGICAL MALIGNANCIES

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From March 1989 to April 2001 129 patients affected by hematological malignancies were treated with hyperfractionated total body irradiation (HyTBI) for the conditioning of SCT. All patients have been treated with HyTBI (3 daily fractions of 120 cGy for 3 days and 2 fractions the forth day, for a total dose of 1320 cGy). For the conditioning regimen in 97 (74%) patients the TBI has been associated with cyclophosphamide 60 mg/m² for 2 days. In 22 patients, other drugs have been associated. In patients characteristics: M 77, F 52; mean age 31 years; median 30; diagnosis: ALL 53, AML 23, CML 30, NHL 13, MDS 3, MM 3, HD 2, Hy Eos 1; disease phase: early 45, advanced 84; kind of transplantation: MUD 67 (3 p 5/6 m/M), IS 14, MDS 3, MM 3, HD 2, Hy Eos 1; disease phase: early 45, advanced 84; kind of transplantation: MUD 67 (3 p 5/6 m/M), IS 14, MOF (1+2), infections 9 (6.9%), TTP 1+1, VOD 1; 34 (27%) patients were in advanced phase and 37 patients were in advanced phase and 37 patients were in early phase. One hundred and eighteen of them have a follow up of 6 months, mean 53 months (range 172-4312 days; median 1267 days). At the present time 48 patients are alive; 42 of them are disease-free (20 ALL, 11 CML, 5 AML, 3 NHL, 1 HD, 1 M M, 1 SE) and 6 relapsed (3 AML, 1 CML, 1 NHL, 1 ALL). In our experience HyTBI seems to be a feasible and well tolerated therapy with a low incidence of acute complications and with long-term survival comparable with literature results.

PO263
DUAL ENERGY X-RAY ABSORPTIOMETRY AND QUANTITATIVE ULTRASONOMETRY FOR THE EVALUATION OF OSTEOPENIA AND OSTEOPOROSIS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Bone complications after allogeneic bone marrow transplantation (BMT) include osteopenia, osteoporosis and osteonecrosis, which lead to pain, disability and increased risk of fracture. Major risk factors for transplanted-related osteoporosis are highly aggressive marrow ablative conditioning regimen, cytokine storm at the time of transplantation and post-transplant long-lasting high-dose steroids and cyclosporin-A therapy. In addition, women frequently experience amenorrhea associated with hypoestrogenism after BMT, which can further decrease bone mass density (BMD). BM D was measured by dual energy X-ray absorptiometry (DEXA) at lumbar spine (L1-L4) and femoral neck (Hologic QDR 1000, Whatman, MA, USA) and by quantitative ultrasonometry (QUS) at proximal phalanges of the non-dominant hand (DBM Sonic, Igea, Carpi, Italy) in 35 long-term survivors after HLA-identical sibling BMT (mean age: 29 years, range 13-50). BM D was expressed as T and Z score, that assess standard deviation of patient’s BM D compared with normal values from healthy young adults and age and sex-matched controls, respectively. Osteopenia and osteoporosis were defined by a T score below -1 and -2.5, respectively. At the time of testing (mean follow-up after BMT: 55 months; range: 1-10 years), serum calcium, phosphorus and alkaline phosphatase, urinary calcium excretion, thyroid and parathyroid hormones, hypopituitary, gonadotropins and sexual hormones (in some cases during replacement therapy) were within the normal range in all patients. Lumbar spine (T and Z score mean value: -0.9 and -0.8, respectively), femoral neck (-1.3 and -1.0, respectively) and proximal phalanges (-1.6 and -1.4, respectively) BMD were significantly reduced in comparison with BM D of 100 healthy controls (p<0.001 for both T- and Z-score in all examined sites). QUS detected BM D reduction in all patients after allogeneic BMT (48% and 20% of cases with osteopenia and osteoporosis, respectively) whereas DEXA documented BM D decreased in 72% and 80% of patients at lumbar spine and femoral neck, respectively (25%/8% and 35%/12% of cases with osteopenia/osteoporosis for lumbar and femoral sites, respectively). Six out of 8 patients who developed osteonecrosis showed QUS values < -1. By QUS, a significant correlation was found between length of amenorrhea and BM D decrease (p=0.01). By QUS and DEXA, BM D was significantly reduced after allogeneic BMT, as demonstrated by DEXA and QUS; 2) as QUS is a safe (there is no radiation load on the patient) and easy method, it should be frequently used for early recognition of BM D reduction and for decision-making in allotransplanted patients who may need hormone replacement and biphosphonate therapy.
We evaluated post-transplant chimerism in 10 subjects submitted to allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning regimen. The patients were affected by myelodysplastic syndrome (MDS) (1 case) acute myeloid leukemia (AML) (6 cases, 1 AML-MDS and 1 therapy-related), acute lymphoblastic leukemia (1 case), non-Hodgkin lymphoma (2 cases). The median age was 44 years (range 24-54); all patients were poor candidates to standard procedure due to age or poor performance status. Conditioning regimen consisted of thiopeta 5 mg/kg/day × 2 days and fludarabine 25 mg/m²/day × 5 days; three patients with overt leukemia received a single dose of idarubicin 15 mg/m² at day -12. GvHD prophylaxis was performed with Cyclosporine A(CsA), methotrexate and methylprednisolone. Chimerism evaluation was performed on peripheral blood samples at the engraftment, at day +30, +60, +90, +180, +360 post-transplant, by microsatellite PCR (loci D3S1349, FGA, D12S67, D19S246, D19S253, D20S85, D2S165, D2S160, D2S367, D2S125, D2S206, D2S117) and ABI GeneScan Analysis. Seven of 10 patients showed complete donor chimerism at engraftment, while 3 patients demonstrated mixed chimerism with a percentage of host cells ranging from 2 to 3.5%. One of them reached complete donor chimerism at day +30 post-transplant, while 2 patients affected by refractory AML without signs of acute GvHD demonstrated a progressive expansion of host cells at day +30 and +60; they underwent immunosuppression discontinuation and donor leukocyte infusion without improvement. One patient with AML had a transient phase of mixed chimerism 120 days after transplant and achieved complete donor chimerism after CsA reduction. Our preliminary data confirm that chimerism analysis has a pivotal role in the management of patients submitted to allogeneic transplant with reduced-intensity conditioning regimen for malignancies where the achievement of a complete donor chimerism seems to be an essential goal. Thiopeta-fludarabine is a well tolerated conditioning regimen with selective hematological toxicity inducing high levels of donor chimerism.

PO265
MONOCLONAL AND OLIGOCLONAL GAMMAPATHIES AFTER ALLOGENEIC BONE MARROW TRANSPLANT

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Background. Several blood protein disorders (e.g., paraproteins) occurring after bone marrow and solid-organ-transplantation are reported. It is known that there is an association between this alteration of the normal immune response and the Epstein Barr virus infection. The relationship between Cyto-megalovirus (CMV) reactivation and such paraproteins is more difficult to establish. Due to the absence of viral genome in the cells it is hard to prove molecular interaction between CMV and B lymphocytes. Methods. We studied 33 consecutive patients who underwent BMT for hematologic malignancies between November 1996 and November 2000. We analyzed the serological status for the commonest herpesvirus in all the donors and the recipients. After BMT, blood samples were evaluated for the identification of paraproteins, performed with zonal capillary electrophoresis and immunofixation. Furthermore we controlled the reactivation of the commonest herpes viruses with pp65 antigenemia for CMV and with qualitative PCR for EBV. The chi-square Test (with Yates correction) was used for the statistical analysis. Results. Electrophoretic anomalies were found in 15/33 (45%) patients: 8 IgG, 5 IgA, 2 IgM. Evidence of pp65+/CMV reactivation was detected in 21/33 pts (63%). In 13/15 cases the oligoclonality was strictly associated with the CMV reactivation (p<0,025). In two patients we observed the reactivation of both EBV and CMV. The median interval between CMV reactivation and paraproteins appearance was 95 days. None of these gammapathies transformed into post-transplant lympho-proliferative disorders (PT-LPD). In all cases oligoclonality is still present with a median follow-up of 25 months (range 6-53 months). Conclusions. Oligoclonal and monoclonal immunoglobulins are frequently observed after BMT and they are statistically associated with a CMV rather than with EBV reactivation. It is a benign phenomenon.
Early engraftment Late engraftment
Cell injected N° pos. % CD45+ Human N° pos. % CD45+ Human
Cell N° pos. % CFC/mouse CFC/mouse
40,000 Unmanip 3/9 0.38 757 1/5 1.14 1068
Amplif 10/27 0.08 2457 2/12 0.26 5263
75,000 Unmanip ND ND ND 4/5 8.27 17581
Amplif 15/18 0.51 863 8/11 3.18 4263

**PO267 LOST EFFECTIVENESS OF RECOMBINANT ERYTHROPOIETIN-EPO THERAPY IN ALLOGENIC BONE MARROW TRANSPLANTATION: SOME COMMENTS ABOUT DELAYED THERAPY**

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Background and objectives. Inadequate EPO production seems to have a fundamental role in the delay of erythrocyte recovery, especially starting from the 3rd and 4th week after allogeneic transplantation. GVHD and CyA and alkylating agent therapy seem to be the cause of inadequate EPO production. Several papers demonstrated that rHuEPO therapy can accelerate erythrocyte recovery and reduct transfusional requirements. In this paper we investigate long terms benefits of rHuEPO therapy. Patients' characteristics: 20 patients were studied: median age 30 (16-58) years; M/F ratio 11/9; average follow-up 360 (72-1020) days; M RD 13/20 (65%) and M UD 7/20 (35%); AB0 compatible 11/20 (55%) and AB0 incompatible 9/20 (45%); acute GVHD (I-IV) 13/20 (65%) and chronic GVHD 14/20 (70%); CMV infection 3/20 (15%). Design and methods. EPO levels were measured from day 22 post BMT. This value is defined to be adequate if included in 95% confidence limit of the regression formula log(EPO) = 3.967 – (0.0695 × HCT) with HCT values between 20% and 40%. Four patients with inadequate EPO production were treated with rHuEPO starting in different time (30-125 days post BMT) after inadequate EPO production evidence. Results. Nineteen patients (95%) had inadequate EPO values (8/13 - 61% - also 6th month post-BMT). The only two differences between the two groups (patients with adequate EPO levels vs. patients with inadequate EPO levels) were AB0 compatibility (14% incompatible vs. 62% incompatible) and the presence of acute GVHD (III-IV) (13% vs. 43%). The comparison between the 9 patients with inadequate EPO levels (not treated with rHuEPO) and the 7 with adequate EPO levels hadn’t shown significative statistical differences in platelet recovery (pit. > 20000/m3: 28.1±17.8 days vs. 18.2±4.4 days, p = NS). In the 9 patients with inadequate EPO levels erythrocytary recovery (HCT > 30%: 211±74.4 vs. 88±66.4 days, p < 0.05) were delayed and transfusional requirements (14,6±8 vs. 4.2±6 RBC units, p < 0.05) were higher than other patients. Erythrocytary recovery was delayed in the 4 patients treated with rHuEPO (HCT > 30%: 545±200 vs. 211±74.4, p < 0.05). Conclusions. In previous works rHuEPO therapy always started within 1 week post BMT. In our patients follow-up was longer (460-1020 days) than in previous works and it was evidenced that the effectiveness of rHuEPO...
therapy was lost as it was stopped. A first hypothesis to explain this evidence is that late rHuEPO administration might not find out an appropriate number of BFU-E and CFU-E reinfused. So must be waited for the differentiation of new BFU-E and CFU-E out of donor stem cells (ca. 4 weeks post BMT). Another hypothesis is that long term rHuEPO therapy could determine a decrease of BFU-E and CFU-E receptors and/or decreased kidney EPO production. It is left to prove that rHuEPO therapy started within 1 week post BMT is more effective in granting erythrocyte recovery in a long-term follow-up.

PO269
ALLOGENEIC BLOOD STEM CELL AND BONE MARROW TRANSPLANTATION FOR HIGH-RISK ACUTE LEUKEMIA: A SINGLE CENTER EXPERIENCE

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We retrospectively analyzed the outcome of 68 pts with high-risk acute leukemia, defined as ≥ 1 CR, cytogenetic abnormality including Ph, 11q- (t(6,9)), hyperdiploidy, or secondary leukemia, who received an HLA-identical sibling BM or PBSC. The clinical characteristics of the two groups are reported in Table 1. Conditioning regimen consisted in the BUCY 4 or BUCY 2 association in all. As GVHD prophylaxis CSA or CSA + MTX was employed in the two groups (p = ns). As a graft, a median of 9 × 10^6/kg CD34^+ and 2.5 × 10^9/kg CD3^+ cells were infused in PBSC pts, while BM pts received a median of 4.6 × 10^6/kg CD34^+ and 0.2 × 10^9/kg CD3^+. Results. Recipient of PBSC recovered ANC > 0.5 × 10^9/L, in a median of 13 days, while BM recipient recovered ANC > 0.5 × 10^9/L in 17 days (p = 0.004). Also in platelets recovery a significant difference was observed (17 vs 22 median, p = 0.007). There was no significant difference in the incidence of aGVHD ≥ II (p = 0.2). The incidence of cGVHD was 75% at one year in the PBSC group vs 40% in the BM (p = 0.001) with a higher incidence of the extensive form in the PBSC group (p = 0.003). No relationship was observed between the median dose of CD34^+ and CD3^+ cells infused and the presence of cGVHD in the PBSC group. Transplant related mortality (at one year) was 6% in the BM group and 23% in the PBSC (p = 0.06). No difference were observed in DFS and OS between the two groups (p = 0.2). At a median follow up of 41 months (range 1-105) in the BM group, 22 pts. are alive and disease free (67%), while in the PBSC group at a median of 13 months (range 1-78), 21 are alive and disease free (57%). In this study it seems that the source of stem cells does not confer any advantage in terms of leukemia recurrence in high risk pts. Although PBSC results in a shorter hematological recovery and hospital stay it is worth considering the impact of severe cGVHD on the quality of life. Further study and better strategies for the prophylaxis and treatment of GVHD are warranted.

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>BM N=32</th>
<th>PBSC N=36</th>
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<tbody>
<tr>
<td>Age median (range)</td>
<td>27 (5-82)</td>
<td>36 (5-57)</td>
</tr>
<tr>
<td>Cytogenetic risk: Low</td>
<td>20/9</td>
<td>13/10</td>
</tr>
<tr>
<td>AML</td>
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<td>19</td>
</tr>
<tr>
<td>ALL</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Secondary Leukemia</td>
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<td>2</td>
</tr>
<tr>
<td>Status 1st CR/advanced</td>
<td>19/12</td>
<td>14/21</td>
</tr>
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</table>

PO270
STANDARD VS ALTERNATIVE MYELOABLATIVE CONDITIONING REGIMENS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR HIGH-RISK ACUTE LEUKEMIA

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From October 1986 to February 2000, at the Institute of Hematology of “La Sapienza” University, 104 consecutive patients (male: n = 63; median age: 21, range 1.3-44.2 years) with high-risk acute leukemia underwent a non T-cell depleted graft from an HLA-identical sibling following a standard or alternative myeloablative conditioning regimen. Of the 104 patients, 60 were affected by acute lymphoblastic leukemia and 44 by acute myeloid leukemia; the phase at transplant was complete remission (CR) > 2nd in 76 pts, untreated 1st relapse with < 20% blasts in 11, refractory leukemia or overt resistant relapse in 17. Conditioning consisting of either 12 Gy fractionated total body irradiation (FTBI) or 16 mg/kg busulphan combined with 120 mg/kg cyclophosphamide were defined standard regimens (n = 38), whereas all other myeloablative regimens (FTBI-60 mg/kg etoposide and three-drug combinations) were considered alternative (n = 66). As concerns patient characteristics, no significant difference was observed between the two groups. Outcome was evaluated by uni-factor analysis and Cox proportional hazards regression model. All patients engrafted and no difference in terms of acute and chronic graft-versus-host disease (GVHD) incidence and severity was observed between the two treatment groups. Sixty-six patients died, 38 of whom of relapse, 26 of transplant-related deaths and 2 of other causes. Thirty-eight patients are surviving with a follow-up ranging from 0.7 to 13.8 years (median, 7.1 years); only one patient is alive 5.7 years after relapse. At the median follow-up, the actuarial probabilities of overall survival, relapse and transplant-related mortality for patients conditioned with standard and alternative regimens were 52% (95% CI, 36-68%) vs 25% (13-37%) (p < 0.02), 34% (18-51%) vs 58% (43-73%) (p < 0.04) and 25% (9-40%) vs 32% (19-44%) (p = ns), respectively. Cox analysis confirmed that, after adjusting for diagnosis, leukemia phase, duration of 1st complete remission and GVHD prophylaxis, alternative regimens were associated with a significantly worse survival (hazard ratio 1.8; p < 0.04). From this retrospective analysis we can conclude that the alternative conditioning regimens we used did not improve the outcome of patients transplanted for high-risk acute leukemia.
The greater potential benefit of allografting for patients with advanced lymphomas could be exploited if conditioning mortality could be decreased and tumour burden minimized before conditioning. One method of achieving this would be to use high-dose therapy (HDT) and autografting to debulk lymphoma, followed by autografting using a nonmyeloablative conditioning regimen. Between June 1997 and January 2001, 34 patients with Hodgkin’s disease (HD) (n=19) and non-Hodgkin’s lymphoma (NHL) (n=15) 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. After a short time, the patients were treated with immunosuppressive therapy consisting of fludarabine 30 mg/m² with cyclophosphamide 300 mg/m² daily for three days followed by the infusion donor-mobilized hematopoietic stem cells. Complete (n=24) and mixed (n=8) chimerism was achieved in 32/34 (94%) patients. Patients with mixed chimerism or with complete chimerism and residual lymphoma, received donor lymphocyte infusion. At a median time of 400 days (range, 60–1217 days) from mini-allografting, 21 (62%) patients are alive. Ten of these patients are in complete remission and two are in good partial remission. Thirteen patients died, eight of progressive HD/NHL, one of progressive HD combined with extensive chronic GVHD, two of infection and two of GVHD. A good correlation between GVHD and response was found.

PO272
DETECTION OF DENDRITIC CELLS (DC1 AND DC2) SUBSETS IN BONE MARROW AND GRANULOCYTE-COLONY-STIMULATING FACTOR PRIMED PERIPHERAL BLOOD HARVESTS FROM ALLOGENIC STEM CELL DONORS

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In peripheral blood (PB) and in bone marrow (BM) two sub-sets of dendritic cells (DC) have been identified: DC type 1, myeloid, are lineage negative, HLA-DR positive and CD11c positive; DC type 2, lymphoid, are lineage negative, HLA-DR positive and CD123 positive. DC1 activate T lymphocytes, while DC2 seem to induce antigen-specific tolerance. Since the number of infused DC can influence the kinetics of the immune system reconstitution and the development of graft-versus-host disease, we counted DC1 and DC2 in the PB and in the BM of allogeneic stem cell donors using a 3-color flow cytometric assay. DC were identified in lysed whole blood or bone marrow as lineage negative (CD3, CD16, CD56, CD14, CD19, CD20), HLA-DR positive, CD11c positive (DC1) or CD123 positive (DC2). In PB the mean number (per microliter) of DC1 was similar to that found in PB before G-CSF administration (3.4±0.1 versus 3.3±0.2, p=0.03); the mean number of DC2 was also significantly increased after G-CSF administration (3.4±0.1 versus 20±0.2, p=0.002). In BM the mean number of DC1 was similar to that found in PB before G-CSF administration (4.1±0.1 versus 4.2±0.2, p=0.94), while the mean number of DC2 was higher than that found in PB before G-CSF administration (3.4±0.1 versus 16±0.2, p=0.06) but similar to that found in PB after G-CSF administration (20±0.2 versus 16±0.2, p=0.88). BM harvests (n=6) contained a mean of 0.04±0.01 x10⁵/kg recipient DC1 and 0.31±0.01 x10⁵/kg recipient DC2 while PBSC harvests (n=8) contained a mean of 0.3±0.01 x10⁵/kg DC1 and 1±0.1 x10⁵/kg DC2. In conclusion, in standard condition the mean number of DC1 is similar in PB and in BM, while the number of DC2 is higher in BM than in PB. However, G-CSF administration increases the number of DC, in particular of DC2 and, for this reason, the number of DC1 and DC2 infused is higher in PBSC harvest in comparison with BM harvest.

PO273
DETECTION AND QUANTIFICATION OF MIXED CHIMERISM FOLLOWING BONE MARROW TRANSPLANTATION USING SINGLE STRANDED CONFORMATION POLYMORPHISM

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Persistence of recipient-type hematopoietic cells after bone marrow transplantation (BMT) is referred to as mixed chimerism (MC). The proportion of donor versus recipient cells can remain stable or shift over time and MC is associated with late graft failure and disease recurrence. The extent of MC can be studied by several methods. PISH analysis has the disadvantages of being only applicable when the donor and recipient are of different gender. While highly polymorphic and sensitive analyses of VNTR and microsatellites have the disadvantages that the smaller allele of a heterozygote is often preferentially amplified. This bias risks both not detecting chimerism and incorrectly estimating the extent of chimerism. Traditional Southern blot analysis does accurately reflect the extent of MC, but it is also time consuming, requires large amount of DNA, and the use of radioactivity. Single stranded conformation polymorphism (SSCP) analysis take advantage of the fact that single stranded DNAs of the same length but different sequences have different conformation and mobility. This method is highly sensitive and can detect sequences with only one base pair of difference. Our SSCP protocol detects the common mutations in our β-thalassemia population, the mutation responsible for sickle cell anemia, as well as many other single nucleotide polymorphisms (SNP). Since the PCR product of the mutated and the normal alleles are the same size, they are amplified in direct ratio to the initial DNA concentration. Band intensities, which reflect the relative amount of DNA originated from donor and recipient cells, are then quantified using image analysis software. In a series of 11 patients, we detected 2 cases (18%) of MC not detected by microsatellite analysis, even though more than one locus was informative. Therefore we propose that SSCP is a more reliable method to detect and follow the evolution of MC. Given the high number of SNP available, it is relatively easy to identify a panel of SNPs that can be analyzed under one or two SSCP conditions and have a combined high heterozygosity for every population.

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non-myeloablative conditioning. The definite graft rejection occurred; aGVHD was seen in 42% of patients. Regimen related toxicity is low; no fatal graft rejection occurred; aGVHD was seen in 42% of patients. The pattern of immune reconstitution was also investigated. At day +90, mean (±SE) values were: CD3+ 714.8±225.8, CD19+ 47.1±31.3, NK 69.9±26.4, CD4+ 256.5±73.5 and CD8+ cells 460.8±203.1. After a median follow-up of 156 days (range, 65-464) overall survival (OS) was 87%; six pts. achieved complete remission (75%) with full donor chimera, 1 pt. relapsed 14 months after transplantation without missing donor chimerism. The TRM at 15 months was 12%. These preliminary results show that non-myeloablative conditioning is well tolerated, has a low risk of TRM and is able to ensure sustained engraftment. The definite graft rejection occurred; aGVHD was seen in 42% of patients. Although responses (CRs and PRs) have been seen longer follow up is needed to assess the role of nonmyeloablative allografts.

In our Center patients not eligible for standard allogeneic transplants because of age or medical contraindications, having an HLA-identical sibling available, were candidates for an allogeneic stem cell transplant using a non-myeloablative conditioning regimen. The basic conditioning regimen included fludarabine 30 mg/m2 on day -4, -3 and -2, and low-dose TBI (200cGy) and postgrafting immunosuppression was with a combination of mycophenolate mofetil (MMF) and cyclosporine (CSA). One patient with metastatic melanoma was conditioned with fludarabine 30 mg/m2 on day -4, -3 and -2, and melphalan 50 mg/m2 on day -3 and -2, with the same postgrafting immunosuppression as described above. Seven patients, median age 57 (range 46-67) years were treated. Diagnosed included MM relapsed after 2 tandem autologous stem cell transplant (n=1), progressive refractory MM (n=1), AML 2nd CR (n=1), AML 1st CR, AML PR (n=1), AML 1st CR (n=1), refractory AML (n=4), Metastatic Melanoma (n=1). Follow-up ranged from 38 to 191 (median 90) days. The patients received unmodified PBSC grafts from HLA-identical sibling, a median of 6.4×10^6 CD34 cells/kg (range 5.0-9.5) were infused. Overall, transplants were well tolerated. Donor engraftment was as follows: on day 28: T cells range 1-91% (median 89); granulocytes 5-99% (median 96%); marrow 5-99% (median 94). Only 5 out of 7 patients were evaluable for donor engraftment at day 56 which was as follows: T cells range 35-99% (median 98); granulocytes 75-99.7% (median 98); marrow range 34-99.7 (median 85%). No fatal graft rejection occurred. Grade II, III, IV acute GVHD occurred in 42%, 0% and 0% of patients, respectively, and responded well to treatment. One out of 3 evaluable patients for chronic GVHD assessment developed cGVHD of the liver. One patient with AML in PR is alive and in CR; one patient with AML 2nd CR rejected the graft and underwent a second NMT from a different HLA-identical sibling using the same conditioning regimen and MMF and CSA postgrafting. The patient well tolerated the second transplant and day 28 donor T cells is 80%; granulocytes 90% and bone marrow 90%; her bone marrow shows complete remission by morphology and flow cytometry. One patient with relapsed MM is alive and in stable PR; one patient with AML 1st CR died of transplant related causes; one patient with refractory MM died of disease relapse causes; one patient with refractory AML relapsed early after NST, she is alive and received 1st donor lymphocyte infusion at day 33; one patient with metastatic melanoma died 123 days after NST of progressive disease. Overall 4 out of 7 patients are alive (57%), 2 are in CR, 1 in stable PR and one has relapsed. In summary non-myeloablative conditioning allows allografting in otherwise ineligible patients (older patients, heavily pretreated or with medical contraindications); regimen related toxicity is low; no fatal graft rejection occurred; aGVHD was seen in 42% of patients. Although responses (CRs and PRs) have been seen longer follow up is needed to assess the role of nonmyeloablative allografts.
It has well known that the incidence of cytomegalovirus (CMV) infection is significantly higher in patients affected by GVHD. Moreover, it has been postulated that CMV could have a central role in the etiopathogenesis of GVHD. In this regard no definitive evidence has supported this yet. In this study, two different diagnostic methods have been applied for the pp65 and pp67 determinations. They represent the CMV matrix tegument proteins which are solely expressed during viral replication and their detection provides a direct correlation with active CMV infection. pp65 and pp67 were detected by an antigenic-assy (clone IC3+AYM Bioline Diagnostico) and by NASBA RNA amplification technology (Organon Teknika), respectively. We analyzed several samples of leukocyte buffy coats in 4 patients undergoing allogeneic bone marrow transplantation, who subsequently developed a grade III-IV acute GVHD of intestine. All samples were positive for both pp65 and pp67. Furthermore, we examined samples from mucous membrane took off, in the same patient, either from histologically normal or histologically proven GVHD area. All intestinal pathological biopsies were positive for mRNA-pp67. On the contrary, samples of biopsies coming from the same patients without any evidence of GVHD were demonstrated to be negative for mRNA-pp67. Finally, no intestinal sample was pp-65 positive. In conclusion, the determination of mRNA-pp67 may be a specific and useful diagnostic tool for evaluating the presence of CMV on histologically proven GVHD lesions. Preliminary results suggest the putative role of CMV in determining GVHD.

PO276
ACTIVE CYTOMEGALOVIRUS INFECTION IN INTESTINAL MUCOSA MEMBRANE LESIONS WITH HISTOLOGICAL DIAGNOSIS OF GRAFT versus HOST DISEASE
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We retrospectively investigated the frequency and the relapse pattern of 103 adult patients with AML (n= 72) and ALL(n=31), who received an allo BMT between November 1990 and June 2001. Preparative regimen included TBI-cyclophosphamide (Cy) in 73 patients, busulphan-Cy in 9, thiotaque-Cy in 10, fludarabine-thiotaque-ATG-TBI in 4, others in 7. GVHD prophylaxis regimen included cyclosporin A (CSA) and methotrexate (MTX) for 85 patients + antithymocyte globulin (ATG) in 18 patients receiving transplants from matched unrelated donors. The median age for AML patients was 38 years (range 18-61). Forty four (61%) were in first complete remission at the time of BMT and 28 (39%) were in advanced stage of disease (> CR1). Fourteen out of 72 patients (19%) relapsed after a median time of 267 (40-944) days (4 in CR1 and 10 in > CR1). All patients had an hematological relapse, except one who had a CNS relapse. The median age for ALL patients was 29 years (range 17-54). Fourteen (45%) patients were in first complete remission at the time of BMT and 10 (55%) were in > CR1. Seventeen out of 31 (55%) patients relapsed after a median time of 322 days (52-937) from BMT (7 in CR1 and 10 in > CR1). Five of these patients (3 in CR1 and 2 in >CR1 at BMT) had an isolated extramedullary relapse, corresponding to 29% of all relapses. Extramedullary sites included: bone (1), pancreas and kidney (1), skin (1) and soft tissue (1), CNS (1), CNS+BMT (2). Most extramedullary relapses were followed by a systemic relapse. In the ALL group 3 out of 5 patients who did not show cGVHD and 11 out of 18 patients with cGVHD relapsed (p=NS). In the AML group 4 out 10 patients who did not show cGVHD and 6 out of 45 patients with cGVHD relapsed (p=NS). Conclusions: there was a non significant trend towards a lower relapse incidence rate among patients with AML who developed cGVHD. Extramedullary relapses after BMT were rare in AML patients even for those in advanced state of disease. In ALL patients we observed frequent isolated extramedullary relapses (29% of all relapses), occurring in unusual sites other than SNC.

PO277
EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION
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Non-myeloablative allografts are associated with decreased transplant toxicity and mortality, but a higher rate of leukemia relapse. Thus, complete chimerism is essential to obtain long-term disease free survival in acute leukemia. Recently, allogeneic stem cell transplantation following reduced intensity conditioning regimens (RI-HSCT) have been performed in patients with acute leukemia, but donor lymphocyte infusions (DLI) were required after transplant in half the patients for persistent disease or relapse. We have transplanted with HLA-matched sibling RI-HSCT 2 acute myeloid leukemia (AML) patients who were not eligible for conventional allogeneic SCT (total mononuclear cells and CD34+ infused: 4.9×10^6/kg and 3.9×10^6/kg and 3.9×10^6/kg and 4.1×10^6/kg and 2.5×10^6/kg, respectively). Conditioning regimen consisted of thiopeta (10 mg/kg) on day -5 and cyclophosphamide (50 mg/kg) on day -3 and -2. GVHD prophylaxis was cyclosporin-A (CsA, 1 mg/kg) and short course methotrexate. The first case was a 46 year old male with secondary AML-M5a showing trisomy 8 and early hyperleukocytosis relapse after 2 chemotherapy courses (GIMEMA/EORTC AML 99P). The second was a 33 year old male with AML-M4 who relapsed 2 year after conventional sibling HSCT and was resistant to 2 chemotherapy courses. Source of stem cells was bone marrow in the first patient and peripheral blood in the second. To enhance minimal residual disease control in these two high risk patients who did not show any GVHD signs after early tapering (day +25) CsA, we performed CD34+ enriched DLI (microallograft) (after donor peripheral blood mobilization with recombinant G-CSF) at day 30 post HSCT(CD3+ and CD34+ cells infused: 0.9 and 0.5×10^6/kg)
in the first case and 1.4 and 0.5×10⁶/kg in the second). Early after first DLI, both patients showed acute GvHD (global grading: III and I, respectively) responsive to steroid treatment (2 mg/kg/die prednisolone and 0.5 mg/kg/die, respectively). So far, both patients are alive in complete remission and with full donor chimerism 6 and 3 months after transplant. We think that prophylactic CD34⁺-enriched DLI following RI-HSCT can be considered as a useful therapeutic option in poor prognosis patients with recurrent or refractory hematologic malignancies.

PO279
UNRELATED DONOR TRANSPLANTATION IN CHRONIC MYELOID LEUKEMIA: HIGH CURE RATE AND LOW TRANSPLANT RELATED MORTALITY

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If new molecules inhibiting tyrosine kinase activity of protein encoded by BCR-ABL are shown able to prolong life in patients with CML, the role of transplantation will have to be reevaluated, especially in patients without a related donor or at high transplantation risk. However, at the moment, cure is the chief objective, hematopoietic stem-cell allotransplantation remains the primary indication and it has to be balanced only against the procedure risks. We report on data of unrelated donor transplants performed in CML in the Bone Marrow Transplantation Unit of Niguarda Hospital in Milan. Twenty-eight unrelated donor transplants were performed in 27 patients. Thirteen were CML (chronic phase 8, accelerated phase 4, blastic crisis 1), 9 were ALL (advanced phase 3), 2 were AM L in advanced phase 2, 2 were myelodysplastic syndromes (1 underwent a second transplant after relapse) and 1 was multiple myeloma. All were nursed in LAF rooms, with a policy of complete sterility. Conditioning regimen was standard CY-TBI (in 25/27); GvHD prophylaxis was CSA (starting day–1) plus short term methotrexate. No ATG was added in 27/28 transplants. Results. Twelve of 13 patients (92%) transplanted for CML are alive with a Karnofsky score of 100%, at a median follow-up of 35 months after transplant. Twenty-one out of 26 patients (81%) transplanted for CML in the chronic phase are alive with a Karnofsky score of 100%, at a median follow-up of 35 months after transplant. TRM is 8% (one early death for liver failure) and the sensitivity of the assay was 1%. Fifteen patients, different malignancies (3 AML, 3 ALL, 2 NHD, 1 HD, 2 MM, 1 CML, 1 DM S, 1 Burkitt Lymphoma, 1 metastatic breast cancer), median age 45, were evaluated for chimerism after allo graft. At transplant only 3 pts were in CR. As conditioning all received TT 5 gr/kg day 1 + FAMP 5×25 mg/kg/day 1-5. CSA alone was employed as GVHD prophylaxis. As graft mobilized PBSC from their HLAd sibling were infused (median CD34⁺ 8.9×10⁹/kg and CD3⁺ 2.1×10⁹/kg). All pts engrafted. The chimeric status was assessed in serial samples at two-week interval starting two day after graft. Our data show that in 5/15 (33%) pts achieved full donor chimerism (FC) with the graft, 4 showed FC at all time points and 1 became MC at 6 months then relapsed. Ten out of 15 (66%) pts presented MC on day 30 posttransplant the percentage of donor T cell (CD3⁺) ranged from 5% to 60%. Subsequent observations showed that 6 pts with <50% donor amount relapsed or showed autologous reconstitution; 2 pts with >50% donor showed transient MC and finally achieved full chimerism at day 60 and 180 respectively. One patient exhibited stable 50% donor DNA but was lost for TRM at 3 months. The quantitative assessment of chimerism may be useful to identify pts headed to relapse or reject, moreover study focusing on lineage-specific chimerism analysis will improve the management and the immunomodulation of the transplant.

PO280
QUANTITATIVE ASSESSMENT OF CHIMERISM AFTER UNRELATED PERIPHERAL BLOOD STEM CELL (PBSC) WITH REDUCED CONDITIONING REGIMEN

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Reduced conditioning regimens (mini), with tolerable toxicity, allow us to offer the allogeneic transplant to patients with poor performance status or advanced age. The presence of mixed chimerism (MC) after transplantation could help to establish graft-host tolerance and to prevent GvHD. In contrast with the application of mini approach is somewhat problematic due to the risk of transplant rejection and disease progression. The study of myeloid and lymphoid chimerism is necessary to monitor and manipulate the engrafment by modulating the patient’s immunosuppression and by donor lymphocyte infusion (DLI). We studied the incidence of MC, in bone marrow and peripheral blood samples after mini allogeneic PBSC transplantation. The assessment of chimerism was performed by polymerase chain reaction (PCR) amplification, short tandem repeat (STR) with fluorescence based detection (GeneScan ABI PRISM 310, PE), from DNA of unselected cells and selected T-cells. Informative recipient and donor specific alleles were obtained in all patients and the sensitivity of the assay was 1%. Fifteen patients, with different malignancies (3 AM L, 3 ALL, 2 NHD, 1 HD, 2 MM, 1 CML, 1 DM S, 1 Burkitt Lymphoma, 1 metastatic breast cancer), median age 45, were evaluated for chimerism after allograft. At transplant only 3 pts were in CR. As conditioning all received TT 5 gr/kg day 1 + FAMP 5×25 mg/kg/day 1-5. CSA alone was employed as GVHD prophylaxis. As graft mobilized PBSC from their HLAd sibling were infused (median CD34⁺ 8.9×10⁹/kg and CD3⁺ 2.1×10⁹/kg). All pts engrafted. The chimeric status was assessed in serial samples at two-week interval starting two day after graft. Our data show that in 5/15 (33%) pts achieved full donor chimerism (FC) with the graft, 4 showed FC at all time points and 1 became MC at 6 months then relapsed. Ten out of 15 (66%) pts presented MC on day 30 posttransplant the percentage of donor T cell (CD3⁺) ranged from 5% to 60%. Subsequent observations showed that 6 pts with <50% donor amount relapsed or showed autologous reconstitution; 2 pts with >50% donor showed transient MC and finally achieved full chimerism at day 60 and 180 respectively. One patient exhibited stable 50% donor DNA but was lost for TRM at 3 months. The quantitative assessment of chimerism may be useful to identify pts headed to relapse or reject, moreover study focusing on lineage-specific chimerism analysis will improve the management and the immunomodulation of the transplant.
Large granular lymphocyte leukemia (LGL) is a slow, chronic and generally asymptomatic disease that is caused by monoclonal T or NK-cell proliferation. It has often been found associated with autoimmune disorders. Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system with a progressive and chronically disabling clinical course. We report a 57-year-old patient with primary progressive multiple sclerosis (MS), later diagnosed with LGL CD3+, and treated with allogeneic bone marrow transplant (BMT) from an HLA-identical sibling. Pre-BMT neurologic evaluation revealed distal hypostenia of the upper extremities, parieto-ataxic ambulation, diplopia, right eye scotoma. The expanded disability status scale (EDSS) was 6.0 (at intervals the patient required assistance in walking 100 meters without stopping). Magnetic resonance imaging (MRI) showed multiple lesions of periventricular, occipital, frontal and temporal white matter, pons and corpus callosum. These lesions did not enhance following gadolinium administration. A conditioning regimen with fludarabine 25 mg/m² and cyclophosphamide 120 mg/kg was followed by allogeneic bone marrow transplant from an HLA-identical brother. Graft-versus-host-disease (GVHD) prophylaxis consisted of cyclosporine and short-methotrexate. Allogeneic engraftment was documented on day +10. Acute GVHD (grade II) occurred on day +16 and was successfully treated with low dose of steroids. At 3 months post-BMT the patient is in complete molecular remission of LGL. Neurologic evaluation showed improvement in motor impairment with an EDSS of 5.5 (the patient could walk for 100 meters without assistance). MRI showed improvement in motor impairment with an EDSS of 5.5 (the patient could walk for 100 meters without assistance). MRI revealed a stable pattern with no new lesions. Continued follow-up is necessary to evaluate the duration of clinical and MRI stability or improvement.

PO281
ALLOGENEIC BONE MARROW TRANSPLANT FOR LARGE GRANULAR LYMPHOCYTE LEUKEMIA IN A PATIENT WITH MULTIPLE SCLEROSIS

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Acute graft-versus-host-disease (aGvHD) is one of the major causes of morbidity and mortality for patients submitted to allogeneic bone marrow transplantation. We retrospectively evaluated 11 patients who received rabbit antithymocyte globulin (ATG- Fresenius S) after an initial acute GvHD resistant to corticosteroids therapy. In one case an escalation dose of donor lymphocyte infusions (DLI) was the cause of aGvHD. Median age at diagnosis was 38 years (27–55). Eight patients received an HLA match unrelated bone marrow transplantation and 3 received HLA sibling bone marrow transplantation. Five patients were affected by chronic myeloid leukemia, one by multiple myeloma, two by non-Hodgkin’s lymphoma, two by acute leukemia and one by myelofibrosis. The conditioning regimens consisted of big BUCY for sibling transplant and TBI/CY for unrelated transplant. All patient received cyclosporine (3 mg/kg continuous infusion) and methotrexate on day +1, +3, +6, +11 for GvHD prophylaxis. Acute GvHD was graded according to the Glucksberg Staging System. The median time of acute GvHD onset was 28 (19 –39) days for 10/11 patients while it was 32 days in the post DLI patient. All patients had more than one GvHD organ involvement, the median grade was III (II–IV). All were initially treated with methylprednisolone (2 mg/kg) for one week, ATG (5 mg/kg every other days for 5 days) was added for GvHD progression, with increased dose of corticosteroids (5 mg/kg) in 5/11 (45%) patients. ATG treatment was well tolerated and no major complications were observed. Six patients developed positive CMV antigenemia during the ATG treatment. On day +28 days after ATG therapy 5 (45%) patients improved, 6 (55%) did worse and two were treated with a second course of ATG and subsequently improved. Overall, 45% patients died before day 100 while the other patients were progressing to chronic GVHD. Thirty-six percent of patients were alive with a follow up of 27(3–45) months. Although the patient numbers are very small, the ATG therapy was useful and safe for progressing skin and liver acuteGvHD, after the failure of corticosteroid treatment.

PO282
ACUTE GRAFT-VERSUS-HOST- DISEASE: A RETROSPECTIVE ANALYSIS OF 11 PATIENTS TREATED WITH ANTITHYMOCYTE GLOBULIN

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Between July 1999 and November 2000, we performed 27(3–45) months. Although the patient numbers are very small, the ATG therapy was useful and safe for progressing skin and liver acuteGvHD, after the failure of corticosteroid treatment.

PO283
REDUCED INTENSITY CONDITIONING REGIMEN FOLLOWED BY ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH HEMATOLOGICAL AND NON-HEMATOLOGICAL MALIGNANCIES. A SURVEY FROM A SINGLE INSTITUTION

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Background. Allogeneic stem cell transplantation (allo-BMT) is considered the treatment of choice for a number of acute and chronic hematological malignancies and some congenital diseases. However, procedure-related toxicity is considerable. To exploit the graft-versus-tumor potential of allogeneic transplant while improving the safety of procedure, we started a pilot study using a non-myeloablative conditioning regimen in poor prognosis hematological and solid tumors patients. Patients and methods. Between July 1999 and November 2000, we performed a non-myeloablative allogeneic peripheral blood stem cell transplantation in 12 patients (7 males and 5 females) having HLA-identical siblings. Seven patients were affected by hematological disease and five by solid tumor. All patients had more than one GVHD organ involvement, the median grade was III (II–IV). All were initially treated with methylprednisolone (2 mg/kg) for one week, ATG (5 mg/kg in 199
PO284

GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS WITH THYMOLGLOBULIN (SANGSTAT OR ATG FRESENIUS) IN PATIENTS ALLOGRAFTED FROM DONORS OTHER THAN HLA-MATCHED SIBLINGS

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The optimal GvHD prophylaxis regimen in transplants from alternative donors has to be defined, since the procedure has an increased risk of severe aGvHD and TRM. We present a prospective, non-randomized study comparing two different ATG preparations (ATG-S, 10 mg/kg in 5 days, -6 to -2, versus ATG-F, 15 mg/kg in 5 days, -6 to -2) added to the conventional GvHD prophylaxis regimen with CyA and short course MTX in 15 patients with hematological malignancies grafted from alternative donors. The primary endpoint was acute GvHD III-IV. Secondary end points were transplant-related mortality (TRM), relapse and survival. Nine patients received ABDRB1-matched bone marrow (7) or partially matched umbilical cord blood (2) transplants from unrelated donors, 6 patients received bone marrow (1) or peripheral blood stem cells (5) from 5/6 matched related donors. Preparative regimen included TBI-cyclophosphamide (Cy) in 12 cases, TBI-Cy and thiotepa in 2 patients receiving cord blood transplants, while 1 patient received TBI-L-PAM. Table 1 summarizes the main clinical results. Primary causes of death were CNS fun-
(12 Gy). Una recidiva midollare a +8 m fu trattata con lo schema FLAG-IDA. Al 14° giorno all’inizio della chemioterapia, nella fase più intensa aplasia midollare, fu praticato il trapianto di cellule emopoietiche allogeniche della sorella (CD 34+: 3,4 × 10^6/kg). Profilassi della GVHD: ciclosporina 1 mg/kg ev da –1 e Mtx 10 mg g+1, 8 mg g+3 e +6. La riconversione ematologica fu rapida con neutrofili > 0.5 × 10⁹/kg al giorno +14 e Piastrine > a 20.000 × 10⁹/kg al giorno +15. Lo stato chimerico donatore-ricevente fu del 30% a +1 mesi, dell’80% a +3 mesi. Il chimerismo permane a +6 mesi con un rapporto del 90% e 4/100 cellule maschili rilevate con sonde centromeriche per i cromosomi X e Y sui nuclei in interfase. Il trapianto allogenico up-front nella fase aplastica della terapia di induzione rappresenta la semplificazione estrema rispetto al trapianto convenzionale programmatto dopo una chemioterapia ciclica con un regime di condizionamento aggressivo. È una strategia del trapianto che incorpora in un’unica sequenza la terapia citostatica di riduzione e eliminazione delle cellule neoplastiche che fa da ponte alla infusione delle cellule emolinfopoietiche del donatore destinate a rigenerare l’emopoiesi e a esercitare l’effetto GVL. Il trapianto up front nelle recidive elimina la variabile del mancato raggiungimento della RC e l’infusione ritardata può ridurre la morbilità e la mortalità della GVHD, probabilmente, oltre che per l’intervenuta regressione del danno tessutale, anche per un più prostrato chimerismo misto che induce una tolleranza bilaterale (Prigozhina T, Exp Hematol 1999,27,503). Queste caratteristiche lo distinguono dal trapianto non mieloablativo e dal trapianto tandem autologo-allogenico. Il trapianto up-front con infusione ritardata di cellule allogeniche può trovare indicazione nelle recidive di leucemia dopo un precedente trapianto, nelle recidive dopo chemioterapia, nelle leucemie refrattarie e in malattie ematologiche non neoplastiche.

**PO286**

**MOLECULAR REMISSION IN A MULTIPLE MYELOMA PATIENT AFTER A NON MYELOABLATIVE ALLOGENIC TRANSPLANT: PROOF OF PRINCIPLE**

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The presence of a graft-versus-tumor effect after allogeneic transplantation has been well established in multiple myeloma (MM). Unfortunately, MM patients tolerate myeloablative conditioning regimens very poorly. This prompted many investigators to design new transplant protocols with reduced intensity or non-myeloablative conditioning regimens. Though these procedures should still be considered investigational and not yet standard treatment, recent reports have shown greatly reduced transplant related mortality (TRM) with high response rates. However, preliminary data on the achievement of molecular remissions are still lacking. At our center, a 46-year old patient with refractory multiple myeloma was conditioned to transplant with low dose total body irradiation, 200 rads, followed by potent immunosuppression with cyclosporin and mycophenolate mofetil. This transplant approach is a form of cell immunotherapy aiming at tumor eradication by means of a potent graft versus-myeloma evoked by donor T cells. At diagnosis, a patient specific clonal marker was generated based upon the rearrangement of the immunoglobulin heavy chain genes and used for polymerase chain reaction (PCR) detection of minimal residual disease post transplant. Monoclonal paraprotein at the time of transplant was 2300 mg/dL with no Bence Jones proteinuria. Marrow plasmacells were below 1%. As expected, myeloma cells by PCR were detected in both bone marrow and blood. On day 0, the patient received donor peripheral blood mononuclear cells with 19.08 × 10⁹/kg CD34+ cells; 2.718 × 10⁹/kg CD3+CD4+ T cells; 1.08 × 10⁶/kg CD3+CD8+ T cells. The post transplant course was rather uneventful. No blood or platelet transfusions were required. The patient developed grade I skin graft-versus-host disease that resolved spontaneously without specific treatment. Disease status was evaluated at monthly intervals for the first three months, and then every three months for the first year. Since the first month post transplant, the patient started showing a progressive gradual reduction of the disease. Disappearance of the monoclonal paraprotein was attained by immunofixation at four months post transplant, at that time a weak presence of the clonal marker of the disease was still detected by PCR. Molecular remission with no detection of myeloma cells was finally achieved at six months post transplant in both bone marrow and blood. The patient is currently undergoing a normal life, disease free at one year follow up. This report provides direct evidence that molecular remission can be achieved in MM after non-myeloablative allogeneic transplantation.

**PO287**

**TOXOPLASMA GONDII ENCEPHALITIS IN TWO ALLOGENEIC BONE MARROW TRANSPLANT RECIPIENTS: AN EMERGING INFECTION AFTER HEAVY IMMUNOSUPPRESSIVE THERAPY**

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In this report we describe two cases of Toxoplasma encephalitis diagnosed antemortem. Both patients were Toxoplasma positive before BMT and, due to trimethoprim allergy, both were on pentamidine prophylaxis. Case #1. A 16-year old male affected by CML was transplanted from an unrelated donor. Case #2. A 42 day course of MMF (CellCept) was started as 3rd line treatment, and GVHD disappeared. On day +160 the patient was seen in the Emergency Room because of generalized seizures and complete left flaccid hemiparesis, without deficit of sensory system. MRI showed a large right parietal lesion and several small lesions with moderate to evident enhancement, the left side was noted. In 48 hours he became hemiplegic. MRI showed multiple lesions with evident contrast enhancement, consistent with Toxoplasma infection. The patient died in a few days. Autopsy confirmed the diagnosis. Comments. Toxoplasma...
plant programs, and we have extended the age limit to 58 years. Based on these results we have reconsidered our trans-
in patients with myeloid diseases (8 AML, 6 CML, 3 MDS) means
ed by MM, the results substantially improve: 1/17 adverse events
evaluation the 3 toxic deaths, which occurred in patients affect-
group of patients is around 18%, but if we exclude from the
The transplant related mortality in the first 100 days in this
suppressive therapy with a good performance status.
ent 5 patients have extensive chronic GVHD, under immuno-

PO288
ALLOGENEIC BONE MARROW TRANSPLANT IN PATIENTS OVER 40:
REPORT AND ANALYSIS OF RESULTS FROM A SINGLE CENTER

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Allogeneic bone marrow transplant over 40 years is consid-
ered a risky procedure because of high incidence of toxic death. In
this report we analysed a group of 22 consecutive patients who
underwent bone marrow transplant in the same unit between
September 1993 and June 2000. The median age was
51 years (range 43-57), the diagnoses were: 8 AML, 6 CML-CP,
5 multiple myeloma and 3 MDS. All but one received the bone
marrow from an HLA-identical sibling, the remaining patient
from an unrelated donor. Twenty of 22 were conditioned with
FTBI 1200 cG followed by cytoxan 120 mg/kg, 2 patients with
Bu 14 mg+cytoxan 120 mg/kg. GVHD prophylaxis consisted of
short MTX+CSA in 16 cases, CSA alone in the 6 remaining cas-
es. Results. Fourteen patients are alive with a median follow-up
of 42 months (range 7-70). We observed 4 toxic deaths within
the first 100 days (3 patients in patients with MM). Two patients died of
relapse and 2 of late infections more than six months after BM T.
Grade >2 acute GVHD was diagnosed in 8 cases (36%). At pre-
vent 5 patients have extensive chronic GVHD, under immuno-
suppressive therapy with a good performance status. Comments.
The transplant related mortality in the first 100 days in this
group of patients is around 18%, but if we exclude from the
evaluation the 3 toxic deaths, which occurred in patients affected
by MM, the results substantially improve: 1/17 adverse events in
patients with myeloid diseases (8 AML, 6 CML, 3 MDS) means
5.8%. Based on these results we have reconsidered our trans-
plant programs, and we have extended the age limit to 58 years.

PO289
ALLOGRAFTING WITH IMMUNOSUPPRESSIVE CONDITIONING OF
FLUDARABINE-CYCLOPHOSPHAMIDE AFTER AUTOGRRAFTING

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Non-myeloablative conditioning regimens are recently being
used in an effort to minimize conditioning regimen toxicity while
maintaining GVT effects. Since it is likely that immunotherapy will
be more effective in a minimal disease status, we designed a
strategy to follow an AutoSCT with AlloSCT to take advantage of
both the antineoplastic and immunosuppressive effects of
AutoSCT to allow successful allogeneic cell therapy. Sixty-two
patients with a median age of 43 years (range, 16-69), were
treated. Follow-up is at a median of 402 days (range, 15-1381). Diagnoses were lymphomas n=34; (HD=19 NHL=15), solid tumors
n=12: (breast=10 K=11 kidney=1 germ cell=1), leukemias n=7:
(CML=6 AM=1), MDS (n=5) and multiple myeloma (n=4). The
patients were conditioned with an immunosuppressive regimen
consisting of fludarabine 30 mg/m2/d × 3 days with cyclophos-
phamide 300 mg/m2/d × 3 days (Flu-Cy protocol) followed by PBSC
grafts from HLA-identical sibling donors mobilized with G-
CSF. CSP/M TX was given post-transplant to control graft rejec-
tion and GVHD. Donor lymphocytes were given for persistent
mixed chimerism and/or progression of malignancy. Major dis-
ease responses were observed in 25 patients who had measur-
dable disease pretransplant and have had sustained engraftment.
These included 16/28 lymphoma, 2/6 CML (both patients
achieved and maintain molecular remission 29 and 10 months
after), 2/5 RAEB and 5/10 breast cancer patients. Acute GVHD
occurred in 20 (32%) out of 62 patients and in 9 (15%) was of
grade >II. Three patients developed and died of diffuse cGVHD.
Seventeen other patients died of progressive disease between 15
and 1071 days after allografting (breast; n=4; HD: n=4; NHL:
n=3; blastic phase-CML: n=2; M M: =1; kidney: =1; M DS:: =1;
germ cell: =1). These data demonstrate that this novel combined
approach dramatically reduced the acute toxicities of conven-
tional allografting even in highly pretreated patients not eligible
for conventional myeloablative allogeneic transplantation.

PO290
CLINICAL GRADING OF ORAL INVOLVEMENT BY CHRONIC
GRAFT-VERSUS-HOST DISEASE: POSSIBLE CORRELATION
WITH THE OUTCOME

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Between December 1990 and December 2000, 103 adult
patients with hematological malignancies developed chronic
graft-versus-host disease (cGVHD) after receiving HLA-matched
sibling marrow (n=38), sibling PBSC (n=50), partially matched
related PBSC (n=4) or matched unrelated marrow grafts (n=11).
 Cyclosporine (CSA) and short course MTX were administered to
97 patients as GVHD prophylaxis, 6 patients received CSA-MTX
and ATG. cGVHD was graded as limited disease in 39 cases and
extensive in 64 cases. Thirty-seven of 64 (58%) patients with
extensive cGVHD received PBSC and 27 (48%) bone marrow as
stem cell source. Extensive cGVHD was associated with oral
involvement in 52 (81%) cases. We adopted the Oral Mucosa
Rating Scale (Cancer 1992; 69:2469) to quantify the severity of
clinically evident oral mucosal changes with a scale ranging
from 0 to 3 (normal to severe). Of the 52 patients with oral
cGVHD, 18 had mild, 19 moderate and 15 severe changes.
Symptoms were absent in 28 cases (54%), 13 (25%) patients report-
ed mouth dryness, 8 (15%) difficulty in swallowing and 3 (6%)
Mucosal scleroderma and ulcerations were observed more frequently among patients with severe oral involvement (63% vs 6%, p =< 0.0001), whereas erythema and/lichenoid changes were predominantly seen in patients with mild/moderate oral involvement (94% vs 37%, p =< 0.0001). One patient with mild/moderate oral involvement had >2 sites involved (cheeks, tongue, palate) as compared to 6 patients with severe oral changes (n=3 cheeks, tongue, lips; n=3 cheeks, tongue, palate) (p = 0.002).

M. Multiorgan or extensive skin involvement (>50% of body surface area) were associated to severe oral changes in 12 (80%) cases and to mild/moderate oral changes in 17 (46%) cases (p = 0.25). 24 (65%) patients with mild/moderate oral cGVHD and 12 (80%) patients with severe oral cGVHD required double or triple systemic immunosuppressive therapy (p =< 0.04); transplant-related mortality was 12%, 5% and 10% for the three groups respectively (p =< 0.04). In conclusion, oral involvement by cGVHD needs to be carefully evaluated after transplant; our preliminary data suggest that cGVHD might be considered as limited disease even in presence of mild/moderate oral involvement.

PO291
GRANULOCYTIC SARCOMA APPEARED AS A MAMMARY MASS TWO YEARS AFTER AN ALLOGENEIC BONE MARROW TRANSPLANT FOR ACUTE MYELOID LEUKEMIA
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A 28-year old male with a diagnosis of acute myelogenous leukemia standard risk by cytogenetics, received a bone marrow transplant in 1<sup>st</sup> CR from an HLA identical sibling in May 1998. The patient was conditioned with busulfan and cytoxan; aGVHD prophylaxis was performed with cyclosporine (CSA) and a short course of metotrexate (MTX). His post transplant course was quite uneventful and the patient was discharged from his isolation room on day +25. His bone marrow assessment showed full donor chimera by cytogenetics (sex mismatch). Two months later the patient experienced a grade II aGVHD which resolved completely with a short course of steroid treatment. His chronic GVHD assessment showed subclinical cGVHD of the skin. Cyclosporine was tapered off by day +180 with no signs of cGVHD. The patient was followed thereafter on a monthly basis in the outpatient Section of our BMT Unit for the first year. In May 2000, approximately two years after his bone marrow transplant, he came to the outpatient clinic because of a palpable right sub areolar mammary mass. At physical examination the mass was hard, firm, adherent to the muscular fascia. The patient underwent an ultrasound evaluation, which showed a focal lesion with irregular borders of about 3.5 cm, with posterior acoustic absorption. The patient underwent a lumpectomy of the mammary mass and of the local axillary lymph glands in the suspicion of a breast cancer. After evaluation from the pathologist the final diagnosis was granulocytic sarcoma with negative axillary lymph glands. The patient bone marrow showed clinical remission by morphology, flow cytometry and full donor chimera by cytogenetics (sex mismatch). The patient’s has been monitored since thereafter with clinical examination, bone marrow examination and ultrasound. Last assessment of the patient is at 10 months after the surgical removal and is negative by clinical examination and ultrasound evaluation is showing normal tissues with no suspicious lesions; bone marrow evaluation showed complete remission by morphology, flow cytometry and full donor chimera by cytogenetics. In conclusion: bone marrow transplant patients are at increased risk of solid cancers of oral cavity, salivary glands, skin, thyroid, bone/continuous tissues and breast. The presence of a granulocytic sarcoma has to be taken into account in the differential diagnosis of a suspicious mammary lesion after a bone marrow transplant for acute myelogenous leukemia even though the bone marrow shows complete remission by morphology, flow cytometry and by chimerism analysis.

PO292
CYCLOSPORIN INDUCED APHASIA PRECEEDED BY PSYCHOTIC DISORDERS: A CASE REPORT
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Cyclosporin (CSP) causes more neurological complications in hematopoietic stem cell transplant patients than any other drug. Essential tremor is almost always present. Seizures and encephalopathy are the most common problems, but also transient focal findings, most often involving the visual system including occipital blindness, have been reported. Full clinical recovery is the rule only if CSP toxicity is readily recognized and the drug is discontinued and replaced with equivalent immunosuppressive therapy. We report on a case where CSP toxicity caused psycotic and neurological disorders. A multiple myeloma patient, diagnosed in 1999, underwent a non myeloablative allogenic transplant from an HLA identical sibling after failing two autologous transplants. The post transplant course was rather uneventful. On day +65 post transplant, while at home, the patient showed evident signs of disorientation and family members recall her say “I do not exist”. The patient was seen at the emergency room of her hometown hospital showing delirium-like symptoms consisting of severe disorientation with prominent disorders of perception. The symptoms abated after a few hours. Some days later, the patient showed an abrupt onset of motor and sensory fluent aphasia. On day +69, she was admitted and CSP was immediately suspended and replaced with FK506. Upon discontinuation, CSP levels were in the range of 200 ng/mL. Both CT scan and MRI of the brain were not suggestive of encephalitis, cerebrovascular diseases and/or focal abnormalities. No EEG evidence of epileptic activity, seizures and/or focal abnormalities was observed. Diffused slower than normal electrical activity, consisting primarily of pathologic δ and θ waves, unchanged by hyperventilation and/or light stimulation, was recorded suggesting generalized brain suffering. A lumbar
puncture revealed electrolytes, protein, sugar, number and type of cells of the cerebrospinal fluid (CSF) within normal limits. Molecular analyses of CSF for the presence of viral DNA of cytomegalovirus, herpes simplex virus, Epstein Barr virus, human herpes viruses 6 and 8 were all negative. Neurological consultations ruled out metabolic disorders. On admission, blood chemistry and blood counts were normal. In particular, there were no other signs and/or symptoms suggesting CSP toxicities with the exception of neurological problems. The day following the discontinuation of CSP, the patient started recovering from the aphasia and her mental status improved remarkably. The aphasia had almost completely disappeared on day +72 and a repeat EEG was greatly improved. Months after transplant, the mental status of the patient and the EEG recordings are within normal limits. Clinical and laboratory findings, imaging studies, neurological and psychiatric consults combined to define this case as a serious, though very rare, manifestation of CSP toxicity. Clinicians should always be aware of CSP toxicity in the differential diagnosis of serious neurological complications in hematopoietic stem cell transplant patients.

P0293
ALLOGENEIC STEM CELL TRANSPLANTATION IN MYELODYSPLASTIC SYNDROMES AND IN SECONDARY ACUTE MYELOID LEUKEMIAS.
RETROSPECTIVE STUDY OF 9 PATIENTS

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From 1984 9 patients (median age 41, range 16-55 yrs), were submitted to allogeneic stem cell transplantation (SCT) in our Institution. Six were affected by high-risk myelodysplastic syndromes (MDS) and 3 by secondary acute myeloid leukemia (AML). In 7 cases the donor was an HLA-identical sibling, and in 2 cases a matched unrelated donor (MUD). The median interval between the first diagnosis and the transplant was 9 (range 4-39) months. The conditioning regimens were: total body irradiation (TBI) + cyclophosphamide (CTX) in 1 patient, TBI + CTX + etoposide (VP-16) + anti lymphocyte globulin (ATG) in 1 patient, CTX + busulphan (BUS) in 4 patients, CTX + BUS + VP-16 in 1 patient, CTX + BUS + ATG in 2 patients. The source of stem cells was the bone marrow in 4 cases, and the peripheral blood in the other 5 patients. GVHD prophylaxis was performed with cyclosporin (CS-A) and methotrexate (MTX) in 8 patients, and with CS-A alone in 1 patient. Four patients died. The causes of death were: acute GVHD in 1 patient, acute GVHD + infection in 1 patient, multi-organ-failure (MOF) in 1 patient, while the remaining patient died of other causes, unrelated to the hematologic disease. The remaining 5 patients are alive, without evidence of relapse (one of them with chronic GVHD), with a median follow-up of 28 (range: 17-66) months.

P0294
PERIPHERAL BLOOD STEM CELL HARVESTING FROM HEALTHY DONORS USING A DISCONTINUOUS FLOW CELL SEPARATOR

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Transplantation of peripheral blood stem cells (PBSC) rather than bone marrow shortens the duration of pancytopenia after allogeneic transplant, and may reduce transplant-related mortality. Progenitor cells mobilized by G-CSF support rapid and durable engraftment and reconstitution in HLA-identical settings. Infusion of high doses, with an adequate conditioning regimen, overcomes the HLA barrier in mismatched recipients. In this study we describe our results with a mobilization protocol consisting of recombinant human granulocyte stimulating factor (rh-G-CSF) 16 µg/kg/day in two subcutaneous infusions for 6-7 days in 208 related donors (118 males and 90 females). The median age was 38 ±12 range (14-63). We performed 658 leukaphereses to collect the PBSC using a discontinuous flow-cell separator (Haemonetics MCS 3p and Plus). The first leukapheresis was performed on the fourth day of stimulation and another one to three were carried out on consecutive days. Two or four collections were done depending on whether they were required for a matched or mismatched transplants. Each procedure lasted for 317±57 minutes (range 113-647). The mean volume of blood processed was 6676 ±1048 ml (range 2370-11554). The mean ACD-A dose was 591±100ml (range 200-1023). The mean volume of the final product was 168±34ml (range 63-303) with a mean cell harvest of 41.3×10⁹±12.8 (range 15.8-104.6). There were 248×10⁹±145 CD34+ cells (range 23.7-1203) and 6.6×10⁹±2.9 CD3+ cells (range 0.68-19). The median CD34+ cell recovery was 55%. Mild and transient side effects like peri-oral and limb paresthesias were observed and treated by correcting the electrolyte imbalance. Only two procedures were suspended because of a hypocalcemic crisis and a metabolic acidosis, promptly resolved without sequelae for these 2 donors. Single vein access required a central venous catheter only in 4% of collections, collection with Haemonetics cell separator is well tolerated by donors and despite the one arm procedure and the low blood volume processed, we obtained good results.

P0295
ACHIEVEMENT OF FULL DONOR CHIMERISM IN MYELOABLATIVE AND NON-MYELOABLATIVE ALLOGENEIC BONE MARROW TRANSPLANTATION

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The achievement of full donor (FD) chimerism is of prognostic value in patients with hematological malignancies (HM) after BMT. In this study, we retrospectively analysed chimerism status...
in a group of 22 patients affected by HM undergoing either myeloablative (MA)-BMT (11 cases) or non-myeloablative (NM)-BMT, with the aim to compare the kinetics of chimerism status assessed by PCR of VNTR and STR regions. Conditioning regimens mainly consisted of BU/CY2 for MA-BMT and fludarabine-containing protocols for NM-BMT. Furthermore, no patient received donor lymphocytes in this study. Samples of bone marrow or peripheral blood were taken from the recipient and donor prior to transplantation in order to screen for an informative marker, and from the recipient on days +15, +30, +50, +90, +180 and +360. Out of the 8 NM-BMT cases, 2 (25%) resulted FD at day +15 as compared with 0 out of 7 evaluated among the MA-BMT group. At day +30, 2 FD cases were seen among the 8 NM-BMT, while 1 of 6 of MA-BMT cases showed a FD status. A comparable number of patients with a FD status (3/8 NM-BMT versus 2/4 MA-BMT) was detected at day +90. Finally, 50% (2/4) and 80% (4/5) of NM-BMT versus 20% (1/5) and 50% (2/4) of MA-BMT showed a FD status at day +180 and +365, respectively. With the limitation of the low number of cases analyzed, the results of this study showed a tendency towards a development of a speedier FD status in NM-BMT. This observation may be of value for taking into consideration NM-BMT also for patients with a high risk of early relapse or progression.

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PO296

GRAFT-VERSUS-TUMOR EFFECT IN ADVANCED STAGE MYCOSIS FUNGOIDES/SEZARY SYNDROME: THE POTENTIAL CURATIVE ROLE OF MINIALLOGRAFT


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No conventional therapy has been shown to modify the natural history of advanced stage mycosis fungoides/Sezary syndrome (MF/SS). The median survival of stage IV patients is less than 2 years, and only a short lasting complete remission (CR) occurs after autografting. Conventional allogeneic transplantation has been reported to be effective only in single cases. We investigated the feasibility and efficacy of allogeneic stem cell transplantation (ASCt) from HLA-identical siblings after a minimal conditioning regimen, in three patients with advanced stage MF/SS, aged 37 (p1), 48 (p2), and 53 years (p11). All of the patients had received more than three lines of conventional therapy and had suffered multiple pre-transplant Herpes virus infections (1 CMV reactivation, 2 thoracic zooster, 1 EBV-related lymphoproliferative disease and pneumonia). Two cases received a conditioning regimen consisting of two cycles of fludarabine 30mg/m2/day and CTX 300mg/m2/day for 3 days, followed by single dose TBI (200Gy). Patient 1 received 200Gy TBI soon after fludarabine (Seattle protocol) to shorten the pre-transplant phase since he had developed EBV-lymphoproliferative disease 28 days before transplant. GVHD prophylaxis consisted of cyclosporin-A 5 mg/kg b.i.d. p.o. from day 1 to +90 and mycophenolate mofetil 15 mg/kg b.i.d. p.o. from day 0 to +27.

Patients 1 and 2 were evaluable for engraftment on day +28, and showed full donor chimerism upon short tandem repeated analysis on peripheral blood CD8+ cells (>95%); the median duration of neutropenia (<500/m3) was 5 and 3 days, and neither had thrombocytopenia (<20,000/m3). CMV p65 positivity occurred in pt 1 and 3. Pt2 developed EBV-related meningencephalitis that resolved over 1 month, and grade II skin GVHD that responded to 1 mg/kg methylprednisolone. Patient #1 had grade III skin GVHD after the discontinuation of Cy-A, which resolved after the drug was resumed. The evaluable patients achieved complete remission as also shown by cutaneous histochemical evaluation. ASCT after a non-myeloablative conditioning regimen is receiving increasing favour because of the low rate of complications, including infections, hepatic VOD and acute GVHD, It has been supported that many hematological diseases can benefit from this procedure, with chronic lymphoproliferative disorders being generally regarded as the best fit indications. In most of these patients, advanced age and a prolonged disease history make conventional BMT unsuitable, but effective GVHD can control the disease even without an aggressive conditioning regimen. MF/SS has so far been a marginal indication for allograft because such patients have the highest degree of the above-mentioned contraindications and this has masked the possibility of considering the alternative choice of a miniallograft. However, experimental data suggest that MF/SS may be effective in inducing a graft-versus-tumor effect, and the results of our preliminary experience support this hypothesis. Patients with extremely advanced disease experienced a prompt improvement in skin lesions attributable to the early clearance of lymphocyte infiltration, and subsequently progressed to CR unrelated to the conditioning regimen.

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We describe a case of a 50-year old patient with metastatic melanoma (left upper lobe nodular lesion) who underwent, after three cycles of conventional chemotherapy, a non-myeloablative stem cell transplant (NMST) from an HLAlaidentical sibling. Before transplantation, the lung lesion was stable. The patient was conditioned with fludarabine 30 mg/m2 on day -8, -7, -6 and melphalan 50 mg/m2 on day -3 and -2 and postgrafting immunosuppression was with a combination of mycophenolate mofetil (MMF) and cyclosporine (CSA). He received 5.0×10^6 CD34/kg unmanipulated allogeneic stem cells. The patient was discharged to the out-patient clinics on day +3, remained afebrile throughout the transplant, did not require blood or platelet transfusions, and no mucositis, alopecia or VOD was recorded. Weekly chest X-rays (CXR) were performed to follow the pulmonary lesion. Donor chimerism on day 28 was as follows: donor T cells 90%, granulocytes 96% and bone marrow 98%. Donor chimerism on day 56 was as follows: donor T cells 96%, granulocytes 99.7% and bone marrow 99.7. On day +22 he developed grade II aGVHD and he was started on steroid treatment with good response.

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A 55-year-old female with AML standard risk by cytogenetics, in 2nd CR received an allogeneic stem cell transplant using a non-myeloablative conditioning regimen. In our Center patients not eligible for standard allogeneic transplants because of age or medical contraindications, having an HLA-identical sibling available, are potential candidates for an allogeneic stem cell transplant using a non-myeloablative conditioning regimen. The basic conditioning regimen included fludarabine 30 mg/m² on day -4, -3 and -2, and low-dose TBI (200cGy) and postgrafting immunosuppression with mycophenolate mofetil (MMF) and cyclosporine (CSA). The patient received an unmodified peripheral stem cell graft and 7.1×10⁶ CD34 cells/kg were infused. Day 28 donor chimerism assessment showed: donor T cells 75%; granulocytes 84% and bone marrow 84%. Disease assessment showed complete remission by morphology and flow cytometry. The patient chronic GVHD assessment showed sub clinical skin cGVHD, and the patient started cyclosporine taper as per protocol. At day +73 she had a decline in donor T cell chimerism which was 60% and granulocytes 63%, and cyclosporine was rapidly tapered off with no GVHD flare. On day +95 donor T cell chimerism was slightly increased and was 68% and granulocytes 74%. On day +139 she had a rapid decline in white cell count and platelet count. At that point a bone marrow examination was performed which showed bone marrow aplasia and donor T cell chimerism was 10.5%. She was admitted on the ward because of neutropenic fever. The patient rejected her first allograft and a second HLA-identical sibling was available. The patient 155 days after her first nonmyeloablative allograft was conditioned with the same regimen used the first time, receiving postgrafting immunosuppression with a combination of mycophenolate mofetil and cyclosporine. The patient received 2.5×10⁶ CD34 cells/kg. The median granulocyte and platelet nadirs were 100/μL and 23,000/μL respectively. The patient well tolerated the second transplant, with only discomfort related to a catheter-related infection which promptly resolved with antibiotic treatment. Day 28 donor chimerism assessment showed as follows: donor T cells 80%, granulocytes 90% and bone marrow 90%. Disease assessment showed a complete remission by bone marrow morphology and flow cytometry. She developed grade II acute GVHD at day 31 post grafting. In summary, a second allograft from a different HLA-identical donor after a non-fatal rejection using the same nonmyeloablative conditioning regimen and same post grafting immunosuppression as for the first transplant seems feasible, well tolerated, with low toxicity, and, although longer follow up is needed, it might be an option in the setting of non-myeloablative allogeneic transplants.

**PO298**

SECOND NON-MYELOABLATIVE TRANSPLANT FROM A DIFFERENT HLA-IDENTICAL SIBLING IN A PATIENT WITH NON-FATAL GRAFT REJECTION AFTER THE FIRST NON-MYELOABLATIVE TRANSPLANT

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A 75-year-old female with AML standard risk by cytogenetics, in 2nd CR received an allogeneic stem cell transplant using a non-myeloablative conditioning regimen. The patient admitted to the ward because of neutropenic fever. The patient rejected her first allograft and a second HLA-identical sibling was available. The patient 155 days after her first nonmyeloablative allograft was conditioned with the same regimen used the first time, receiving postgrafting immunosuppression with a combination of mycophenolate mofetil and cyclosporine. The patient received 2.5×10⁶ CD34 cells/kg. The median granulocyte and platelet nadirs were 100/μL and 23,000/μL respectively. The patient well tolerated the second transplant, with only discomfort related to a catheter-related infection which promptly resolved with antibiotic treatment. Day 28 donor chimerism assessment showed as follows: donor T cells 80%, granulocytes 90% and bone marrow 90%. Disease assessment showed a complete remission by bone marrow morphology and flow cytometry. She developed grade II acute GVHD at day 31 post grafting. In summary, a second allograft from a different HLA-identical donor after a non-fatal rejection using the same nonmyeloablative conditioning regimen and same post grafting immunosuppression as for the first transplant seems feasible, well tolerated, with low toxicity, and, although longer follow up is needed, it might be an option in the setting of non-myeloablative allogeneic transplants.
counts during G-CSF administration. Fifteen males and 17 females with a median age of 47 years (range 19-68) were submitted to 50 leukaphereses. The processed blood volume target for a single apheresis procedure was 3 times the total blood volume of the donor. All donors had adequate peripheral venous access and all procedures were completed. Almost all donors tolerated the procedures very well, apheresis side effects (paresthesia and dizziness) were promptly resolved by administration of calcium-gluconate i.v. Donor platelet count significantly decreased after procedures but none of the donors requested autologous platelet transfusion. The preapheresis median white cell count was 44,065 per µL (range 22,000-81,270), median platelet count was 199,000 per µL (range 97,000-499,000) and median CD34+ cells per µL was 58.15 (range 12-384.2). The target collection was 5x10^6 CD34+ cells per kg of recipient’s body weight. The median CD34+ cell dose obtained for a single procedure was 5x10^6 (range 23.6-1292) corresponding to 4.6x10^6 (range 0.52-28.1) per kg of recipient’s body weight. Blood cell separator efficiency collecting CD34+ cells was 49.4% (range 16.3-77.3). Twenty-one out of 36 donors (59%) reached the collection target in one leukapheresis while for 15 donors (41%) a second procedure was necessary. The median CD3+ CD19- CD4+ CD8+ cells doses/kg of recipient’s body weight for single leukapheresis were respectively 184.0x10^6 (range 39.5-558), 32.0x10^6 (range 10-139), 129x10^6 (range 26-359), 72x10^6 (range 22-259). In conclusion, a schedule consisting of 4-day administration of G-CSF followed by a single leukapheresis can be proposed and widely accepted by healthy donors, as 59% of them reached the target in the estimated time without serious early side effects. Moreover in our experience, no late side effects were shown after 35 months of follow-up. The search for the optimum methods of donor management may improve the acceptability of this procedure and increase the number of allogeneic transplantations from PBSC.

P0300

ANTI-THYMOCYTE GLOBULIN AND TOTAL LYMPHOID IRRADIATION AS A SALVAGE NON-MYELOABLATIVE PREPARATIVE REGIMEN IN PATIENTS WHO HAVE REJECTED GRAFTS AFTER LOW-INTENSITY CONDITIONING

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As shown by the Seattle group, allografts can be established using a minimally myelosuppressive conditioning regimen that relies on pre- and post-grafting immunosuppression. However, such low-intensity regimens lead to a higher risk of graft rejection, especially in older and heavily transfused patients, those who receive a small number of donor stem cells, or those with advanced stage diseases. We have developed an allogeneic stem cell transplant (ASCT) approach using an immunosuppressive preparative regimen consisting of a single low dose TBI (200cGy) delivered after 2 cycles of fludarabine and cyclophosphamide. Of the first 10 patients treated, two males aged 53 and 54 years experienced graft rejection. Both had stage C B-CLL and had received fewer than 5x10^6 CD34+ cells/kg. They underwent a second allogeneic PBSC transplant 98 and 121 days after the first procedure. The conditioning regimen consisted of fludarabine 30 mg/m^2 on days -14, -13, -12, anti-thymocyte globulin 3 mg/kg on days -4, -3, -2 and 450cGy total lymphoid irradiation (TLI) on day 0; GVHD prophylaxis included cyclosporine-A 5 mg/kg b.i.d. on days -4,-3,-2 and 450cGy total lymphoid irradiation (TLI) on day 0; GVHD prophylaxis included cyclosporine-A 5 mg/kg b.i.d. p.o. from day 1 to +90 and mycophenolate mofetil 15 mg/kg b.i.d. p.o. from day 0 to +27. Short tandem repeated analyses of sorted peripheral blood CD3+ cells showed full donor engraftment by day +14 (>95% donor). After a follow-up of 2 and 11 months, both patients have reached a stable neutrophil count of more than 0.5x10^9/L, but a stable platelet count of more than 50x10^9/L has so far been achieved only in one. Clinically severe adverse events occurred in both cases. At the time of transplant, together with tri-lineage aplasia one patient had multifocal mild pneumonia resistant to amphotericin-B, and then he developed currently almost resolving hemorrhagic pleural and pericardial effusion, ascites and severe hepatic and renal impairment. The other patient was diagnosed as having CMV pneumonia while he was being treated for disseminated aspergillosis; both resolved after treatment. Graft rejection is an expected complication of ASCT after a non-myoeloblate conditioning regimen related to the low intensity of the regimen. However, our small contribution confirms the possible causative role of the other patient- and donor-related risk factors: the two patients were the oldest, had very advanced disease and had received a suboptimal number of donor stem cells. Given the particular clinical profile of these patients, increasing cytoreductive intensity in an attempt to perform a second ASCT may lead to the excess TRM that has previously discouraged the referral of CLL patients for allogeneic BMT. Our experience suggests that sustained donor engraftment and disease control can be achieved by increasing the degree of immunosuppression. This choice is not devoid of risk because multiple aggressive infectious complications may arise as a result of deep immunosuppression and pre-existing pancytopenia, but once successful engraftment has been achieved, graft-versus-tumor alone may effectively control the disease.
IgE levels were evaluated in 17 multiple myeloma patients (10 IgG, 6 IgA, 1 κ) before and after idiotype vaccination with autologous monoclonal component (MC) conjugated with KLH. Baseline IgE levels were already above the 20 U/mL threshold (mean 270±336.7) in 6 patients (35.3%). After vaccination, high values (mean 2411±307) were noted in 12 patients. Another 2 reached values >20 during the treatment and were normalised when it ended. No increase in total IgE was observed in 2 patients. Values >100 U/mL indicative of frank allergy were reached in 8 patients. A 5 and 4 - layer ELISA method was then elaborated to look for specific anti-KLH and anti-MC IgEs. The anti-KLH antibody was purified IgGs from patients who had demonstrated a high IgG response to KLH. The controls were sera of subjects with IgE values similar to those of the patients. The results were expressed as the ratio between the patient and control absorbance values. Five patients displayed values >1.5 (i.e. more than 1.5 times the control values). In addition, 1.5 (as an increase of 50%) was arbitrarily chosen as the response threshold Anti-MC IgEs were sought with autologous MC and a heterologous MC with analogous light and heavy chains. The results were again expressed as the ratio between the patient and control absorbance values. Six patients displayed values >1.5. Determination of the IgEs showed that vaccination increased their levels in 88% of the patients. The search for specific IgEs revealed positivity for KLH in only 29% compared with the observation of anti-KLH IgGs in 92%. Interestingly, none of the patients displayed IgG antibodies against their own MC, whereas specific anti-MC IgGs were noted in 35%, all with IgG MC. IgEs against both autologous and heterologous IgGs were thus produced by 70% of the patients with IgG MC. These results, together with the observation of 3-fold or greater increase in the number of eosinophils in all patients during the first three months of the treatment, point to activation of CD4+ cells in the TH2 subset. As already mentioned, IgE baselines levels were elevated in 35.3% of these patients prior to vaccination. This could point to the presence of a prior TH2-type immune response. The fact that the idiotype vaccination stimulated a response of this type with regard to the MC that was less effective than the TH1 response, whereas anti-KLH IgM and IgG antibodies were produced, means that the method used to prepare the vaccine and/or adjuvants employed in costimulation must be revised.

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**PO301**
DETERMINATION OF TOTAL AND SPECIFIC IgEs IN MULTIPLE MYELOMA PATIENTS AFTER IDIOTYPE VACCINATION

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**PO302**
13q DELETION IN MONOCLONAL GAMMOPATHIES OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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A deletion of the long arm of chromosome 13 (13q-) is detected in 33-55% of MM patients at the onset of the disease and in 70% of relapsed patients. It has been recently observed that 13q- can also be identified in patients with monoclonal gammapathy of undetermined significance (MGUS). However, despite these data the real incidence and nature of 13q- is difficult to estimate as most studies included already treated patients and as cytogenetic analyses were not carried out on selected plasmacells. In a three-year-period we have tested for 13q deletion 18 MGUS and 50 MM patients (10 at disease presentation and 40 during disease outcome). After dilution with phosphate-buffered saline (PBS) bone marrow mononuclear cells were separated by density gradient centrifugation over Ficoll-Hypaque. Subsequently mononuclear cells were washed twice in PBS, fixed in Carnoy’s solution and stored at -20° C. FISH was carried out with the LSI 13 probe, covering the Rb locus and giving a green signal, with the D13S319 and D13S25 probes, both giving red signals. In every patient FISH examination was performed by simultaneously hybridising the LSI 13 probe along with one each of the other two probes. The presence of only one green signal and of only one red signal confirmed FISH hybridisation efficiency, indicating however the presence of monosomy or of a gross chromosome 13 deletion. The cut-off value for D13S319 and D13S25 probes was determined after adding three times the standard deviation to the mean percentage of monosomic cells found in twenty normal controls. Based on this approach the cut-off values were fixed at 5%. A simultaneous loss of the signals due to D13S319 and to D13S25 probes was observed in most patients. Considering the 18 MGUS studied a 13q- was observed in 2 cases. One patient has developed a MM while the other one has just entered the study. In MM 13q- incidence was 34%. The deletion was identified in 5 out of the 10 patients studied at disease onset and in 12 patients examined during the follow-up. Ten of these last 12 cases experienced disease progression with an increase of bone marrow plasmacells or of the monoclonal component or they developed further bone localizations, while the remaining two had a stable disease. In conclusion, 10 of the 19 patients with an unstable disease presented a 13q-. Our findings indicate that no apparent minimal deleted region is present on 13q. Deletions usually begin at least at 13q14 and may involve all 13q. The deletion is not induced by chemotherapy being found in MGUS and in untreated MM patients. Moreover the fact that it has been discovered in 2 MGUS patients might suggest that it is an early event in the development of monoclonal gammapathies with an as yet undetermined role in the evolution toward MM. The presence of 13q seems however to correlate with an unfavorable prognosis.
PO303
CYTOKINES AND MONOCLONAL GAMMOPATHY

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Monoclonal gammapathies are collections of lympho-proliferative diseases characterized by the production of immunoglobulins or their light chain. There might be, at the base of such pathologies, genetically transmitted immunitory defects, for example particular antigenic settlements with similar characteristics to those of some viruses or bacteria, which could be predisposed to clonal development. In several studies, anomalies of the cellular adhesion mechanism and the micro environmental concentration of cytokines are described. The purpose of our study was to evaluate the degree of influence of the single factors and their prognostic index in the evolution of the pathologies. We focused on the cellular adhesion mechanism and the micro environmental concentration of cytokines are described. The purpose of our study was to evaluate the degree of influence of the single factors and their prognostic index in the evolution of the pathologies. We focused on the cellular adhesion mechanism and the micro environmental concentration of cytokines are described. The purpose of our study was to evaluate the degree of influence of the single factors and their prognostic index in the evolution of the pathologies.

PO304
THE URINARY PROTEINS ARE RELIABLE MARKERS OF INITIAL RENAL DAMAGE AND USEFUL TOOLS FOR THE EVALUATION OF TUMOR BURDEN IN MULTIPLE MYELOMA PATIENTS

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Background. Renal failure occurs in about 50% of pts. with MM. It is considered an adverse prognostic factor, particularly if irreversible. By contrast, if treated in early phase, renal dysfunction can be reversed. Urinary microproteins like α1-microglobulin (α1M), a marker of tubular function; IgG and albumin (Alb), indices of glomerular filtration, have been identified as sensitive indicators of renal damage. Patients and Methods. We performed a study on 111 MM pts with median age of 56 years, of whom 30 underwent periodic controls. The aim of the study was: 1) to evaluate the role of urinary proteins in the early diagnosis of renal dysfunction; 2) to elicit their correlation with the indices of tumor burden and with the behaviour of the disease. Results. The incidence of renal damage in our cohort rose consistently from 22% to 64% when urinary proteins, instead of creatinine and ureaemia, were considered. In univariate analysis, α1M and IgG correlated with stage (p=0.01; p=0.0005), uremia (p=0.0007; p=0.007), β2-microglobulin (β2M) (p=0.00001; p=0.007) and bone plasmacytosis (BMPC) (p=0.02; p=0.009); α1M showed correlation also with BJ (p=0.00001) and creatininemia (p=0.002) and IgG with monoclonal component (MC) (p=0.0005). Alb correlated only with clinical stage (p=0.04), BJ (p=0.007) and β2M (p=0.0004). In multivariate analysis IgG was correlated with MC and BMPC, α1M with β2M. Urinary proteins were well correlated with each other and indices of tumor burden (β2M, BMPC, MC) as well. Within the subgroup of pts submitted to periodic controls, the variations of urinary parameters well reflected the behaviour of the disease (improvement, worsening, plateau).

Conclusions. 1) Urinary proteins are useful in the early definition of the extension and the type of renal lesion in MM pts. 2) They correlate well with MC, β2M and BMPC, and with their variations. Therefore, they could help together with the indices of tumor burden, to identify, within asymptomatic MM pts, those who would benefit from an early treatment.

PO305
AMYLOIDOSIS: A RARE DISEASE? SOME DATA FROM THE FLORENCE GROUP

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From the interdisciplinary Amyloid Study Group of the Azienda Ospedaliera Careggi in Florence 23 new cases of amyloidosis (7 males and 16 females) have been detected between December 1999 and November 2000. All patients were Italians of white race, their median age was 66 years (range 32-89 years). They
Low-dose thalidomide plus dexamethasone: an effective treatment in advanced multiple myeloma


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Thalidomide has been recently introduced into the treatment of multiple myeloma thanks to its antiangiogenic and immunomodulatory properties. A pilot study demonstrated the efficacy of thalidomide in refractory and recurrent myelomas with an overall response rate of 32%. The study design called for a gradual increase in the dose, but only 55% of the patients received the intended maximal daily dose of 800 mg because of high toxicity. We investigated the toxicity and clinical efficacy of lower thalidomide doses; as glucocorticoids are effective and extensively used in the management of advanced myeloma patients we also tested the synergistic effect of the association between thalidomide and dexamethasone. To address this issue, between June 1999 and August 2000, 77 patients with advanced myeloma (4 patients were primary resistant to induction treatment, 21 were resistant relapse and 52 were untested relapse) were treated with thalidomide 100 mg/day continuously and dexamethasone 40 mg, days 1-4, every month. After a minimum of 3 months of treatment, 14 patients (18%) showed a myeloma protein reduction 75%-100%, 18 patients (23%) showed response 50-75%, 19 patients (25%) response 25-50% and 26 patients (34%) response <25% or disease progression. After a median follow up of 8 months, median progression-free survival was 12 months. Low dose thalidomide was well tolerated: constipation (12%) and sedation (6%) were mild, tingling or numbness were present in 17% of patients, and discontinuation of the treatment for toxicity was required in 8 patients only (10%). The association of thalidomide plus dexamethasone is effective in advanced myeloma patients even at lower doses. A significant proportion of refractory and relapsed patients benefit from this low-toxicity treatment as a salvage therapy postponing the delivery of chemotherapy.

Multimetric Flow Cytometry Immunophenotyping of Bone Marrow Plasma Cells in Multiple Myeloma and Monoclonal Gammapathy of Undetermined Significance


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Plasma cell (PC) multiparametric immunophenotyping can differentiate between neoplastic cells and their normal counterparts: normal plasmacells are consistently CD19+/CD56-, while myeloma plasma cells are predominantly CD19+/CD56+. Using a flow cytometric technique based on simultaneous triple labeling with CD38/CD56/CD19 and a two-step acquisition procedure, it is possible to enumerate and characterize plasma cells when they represents a few as 0.01% of total bone marrow (BM) cellularity. In the first step 50000 events corresponding to the total of nucleated BM cells were acquired; in the second step only those events included in a live-gate drawn on SSC/CD38 bright fraction (where PC are located) were acquired and studied for the relative expression of CD19 and CD56. We have applied this assay to evaluate the BM plasma cells of 14 patients (pts.) with monoclonal gammapathy of undetermined significance (MUGS) and of 51 pts with multiple myeloma (MM) sub-
PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: FEASIBILITY OF A MIXED INPATIENT-OUTPATIENT MODEL

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Between October 1994 and April 2001, 79 consecutive peripheral blood stem cell transplantations (PBSCT) were performed in 51 MM patients, 35 following a total inpatient model (TIM) and 44 a mixed inpatient-outpatient model (MIOM). Induction chemotherapy mainly consisted of DAV±CTX. All cases were conditioned with melphalan (Mel) 200 mg/m². Twenty-five patients underwent a single (15 TIM, 10 MIOM), 24 a double (8 TIM, 16 MIOM) and 2 a triple (2 MIOM) PBSCT. MIOM cases were older than the other group (49.8±8.1 years in TIM vs 55.5±6.4 in MIOM, p=0.0054), while cases were equally distributed for sex and disease status at transplantation. No difference was demonstrated in terms of stem cells infused (7.2±7.1x10^6/kg in TIM vs 5.5±8.1x10^6/kg in MIOM). The time for granulocyte recovery was shorter in the MIOM group (9.0±0.8 days in MIOM vs 9.5±0.9 in TIM, p=0.0194), while no significant difference was documented in terms of platelet engraftment (13.3±4.8 days in TIM vs 12.7±2.1 in MIOM). Neither the number of grade II-III mucositis (33% in TIM vs 26.8% in MIOM), nor febrile episodes (56.1% TIM vs 76.4% MIOM), nor fever duration (3.8±1.7 days in TIM vs 3.4±1.6 in MIOM) were differently distributed in the two groups. As expected, the median duration of hospitalization was only 4 days (range 2-19) among the MIOM procedures when required, which was significantly shorter than the 18 days of hospitalization in average (range 14-37) for the TIM transplants.

MICOM failed in 17 cases, because of reduced compliance to MIOM in 3 cases, severe reduction of water intake in 2 cases, pulmonary thromboembolic event in 1 case, renal toxicity in 2 cases and sepsis in 9 cases. One case of death was transplant-related in MIOM group at day+12. In conclusion, outpatient management and liberal hospitalization criteria of these patients has resulted in safe conduct of MIOM transplants. Therefore, MIOM can be offered to MM patients in alternative to a TIM program.

PO309
A RETROSPECTIVE EVALUATION OF 39 PATIENTS WITH AL AMYLOIDOSIS FOLLOWED AT A SINGLE INSTITUTION IN THE LAST TWENTY YEARS

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We report a retrospective evaluation of 39 patients, 21 male and 18 female, median age 65 years, (47-83) with AL amyloidosis (24 ± 15 - light chain) followed at our Centre in the last twenty years. Overall 39 patients, 19 (48%) had a biopsy-proven AL amyloidosis, 20 (52%) were diagnosed on the periumbilical (PUF) fat aspirates, which was positive in 34/37 (92%) cases, underwent to this procedure. Three patients, whose PUF produced negative results, had undergone biopsy of other sites that were positive for amyloid (bone marrow, tongue and of soft tissue in a patient with carpal tunnel syndrome). Out of 39 patients, 22 (56%) had a primary AL amyloidosis and 17 (44%) presented associated immunoproliferative disorders: 13 multiple myeloma, 2 Waldenström disease, 1 splenic lymphoma with villous lymphocytes (SLLV), 1 splenic B-cell marginal zone lymphoma (SMZB-cell lymphoma) without villous lymphocytes. The predominant amyloid-related organ involvement was renal in 17/39 (43%) cases, cardiac in 12/39 (30%), neuropathic in 4/39 (10%) and others in the remaining 6 (17%) patients. Eleven patients (28%) presented only one involved organ, whereas 28/39 (72%) two or more. From 1988, when the Italian AL Amyloidosis study group was started, the patients followed at our center, participating in this cooperative group, were treated according to suggested treatment protocols. Two patients, in poor clinical conditions, died before the start of treatment. Five patients with older age, presenting only few mild and stable signs of AL amyloidosis, are closely followed and do not receive at moment any treatment. Two patients are on melphalan and prednisone (MP) for two months (m) and are not valuable for response. One patient was lost to follow-up one month after the diagnosis. Of the 29 patients evaluable for response, 23 (80%) were treated with MP, 2 (7%) underwent to high dose therapy and peripheral stem cell transplantation (PSCT) performed at other center, 3 (10%) received other regimens and one, affected by SMZB-cell Lymphoma underwent to splenectomy. Response was observed in 7/29 (24%) patients (2 MP, 1 splenectomy, 2 PSCT, 2 other regimens). The unresponsive patients received a second and more line treatments with palliative intent and/or supportive therapy. With a median follow-up of 36 m (1-150), 21/39 (54%) patients are alive. The estimated median survival was 56 m. Patients with congestive chronic failure (CHF) at the onset presented a shorter survival (9 vs 69 m, p = 0.0019), whereas a longer survival was shown in the group with only one organ involved (70 vs 52, p = 0.046). In our experience, CHF and the
PO310
MULTIPLE CYCLES OF HIGH-DOSE MELPHALAN AND PERIPHERAL BLOOD STEM CELL TRANSPLANTATION MAY IMPROVE PROGNOSIS IN PATIENTS AFFECTED BY MULTIPLE MYELOMA

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The aim of this study was to analyze the outcome of patients with MM a therapeutic program consisting of high-dose melphalan (HDM) and peripheral blood stem cell transplantation (PBSCT). After induction chemotherapy with VAD, 51 patients with MM, stage II-III, median age 52 years, entered a study consisting of one-three PBSCT. Patients were conditioned with HDM (200 mg/m²). The median number of CD34+ cells was 6.4×10⁶/kg. The median time to recycle HDM was 4 months. All patients underwent at least one PBSCT procedure, 29 cases a double and 2 a triple, for a total number of 79 PBSCT procedures. Considering the 1st cycle of HDM, the median duration of severe neutropenia and thrombocytopenia was 9 ± 13 days, respectively. Forty-one percent of cases experienced at least one febrile episode, mostly of unknown origin. Mucositis was observed in 55.4% of cases (WHO grade 3-4 in 26.7%). No difference was noticed between the 1st and the 2nd PBSCT in terms of hematological and extra-hematological toxicity. Only one patient died because of transplant-related cause. At the 1st PBSCT 27.4% of cases were in CR, 49% in PR, 7.9% in SD and 2.7% in PD. Up to now, CR increased up to 78.4%. In particular, CR was achieved in 93.1% of the 29 patients who underwent 2nd PBSCT. After a median follow-up of 17.8 months from the 1st PBSCT, 38 patients were alive and in CR+PR. At 5 years, overall survival and progression-free survival were 59% and 24%, respectively. In summary, multiple cycles of HDM and PBSCT is a strategy producing a high rate of complete response which is crucial for achieving long-lasting disease control in MM. Partially supported by AIL and Regione Calabria.

PO311
A PILOT STUDY EVALUATING SAFETY AND EFFICACY OF LOW DOSE THALIDOMIDE AND DEXAMETHASONE COMBINATION IN ADVANCED MULTIPLE MYELOMA

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Thalidomide is a recently re-discovered drug with heterogeneous and still not completely clarified biological activities, including immunomodulating and anti-angiogenic capacities, which has been successfully employed for treating advanced multiple myeloma (MM), resulting in a response rate of about 30%. The high dose of thalidomide initially proposed (up to 800 mg/d), however, is often poorly tolerated, especially in elderly patients. Indeed, in our previous experience with thalidomide alone in hematological malignancies, only 30% of patients tolerated doses higher than 200 mg/d, mainly because of significant neurological, gastrointestinal, hepatic and renal side effects. Some recent evidence has also suggested that the activity of thalidomide may be potentiated by addition of steroids. Thus, we planned a pilot study by administering low dose thalidomide in combination with high dose dexamethasone, another drug with well known efficacy in MM, to 15 patients with advanced MM (7 males and 8 females, mean age 68 yrs, range 41-81). All patients but one had received at least 3 lines of chemotherapy and 3 of them had undergone one or more cycles of high dose chemotherapy followed by autologous peripheral blood stem cell transplantation (PBSCT). Dexamethasone was given at the dose of 40 mg i.v. for 4 days every 3 weeks. Thalidomide was given on a compassionate basis, after informed consent was achieved, at the dose of 100 mg/d per os, at bedtime. This dose was doubled (200 mg/d) if no response was achieved after 2 cycles (6 weeks). All patients received at least 12 weeks of treatment and were evaluable for response. Eight patients required the second level dose of thalidomide. Mild somnolence and constipation were observed only in 5 of these subjects. No other significant adverse events were recorded. Five patients did not show any significant modification of the disease after 4 cycles of therapy and stopped the treatment. Three additional patients showed progressive disease after an initial moderate reduction of M-component and were also considered unresponsive. The remaining 7 patients (47%) evidenced a significant response (reduction of M-component > 50% in 5 cases and >75% in 2), associated with a clear amelioration of bone pain, performance status or anemia, and a decrease in marrow plasma cell infiltration. Responses were observed at the thalidomide dose level of 100 mg in 3 patients and 200 mg in 4 patients. Interestingly, 4 responders had relapsed or had been considered resistant after treatments containing high dose dexamethasone. Furthermore, 2 responders had not received significant benefits from previous treatments including PBSCT or thalidomide alone, respectively. Median duration of response was 22 weeks. These findings, although very preliminary, encourage us to evaluate in more details this combination of low dose thalidomide and dexamethasone, suggesting that this association may result in a synergistic effect, with a reduced rate of side effects and good efficacy in heavily treated MM patients with advanced disease.

PO312
FACTORS INFLUENCING COLLECTION OF PERIPHERAL BLOOD PROGENITOR CELLS IN PRIMARY REFRACTORY OR RELAPSED MYELOMA PATIENTS

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Over the past 15 years, a number of clinical trials have been devoted to evaluate high-dose sub-myeloablative chemoradiotherapy requiring hematopoietic stem-cell support in multiple myeloma. It is unclear whether the optimal timing of high-dose
chemotherapy with peripheral blood progenitor cell (PBPC) support is early or late in the course of disease or late. Since an optimal collection of PBPC remains a remains a crucial procedure to perform an high-dose therapy we evaluated several variables that could influence the mobilization an particularly the previous chemotherapy that patients received. Thus, we evaluated 89 patients in relapse phase or refractory to chemotherapy who underwent a standard mobilization procedure with cyclophosphamide and growth factors. All patients were treated according to the same mobilization protocol: CY 3 g/m² followed by G-CSF 10 µg/kg from day 3 to the last day of the leukapheresis. A adequate collection with a median of 3.37 × 10^6 CD34+ cells/kg was defined as a sufficient yield. Thus, patients were delineated in two different groups: 59 patients achieved an adequate collection and 30 did not. The two groups had similar mean clinical features: median age, bone marrow plasmacells and clinical stage. A reliable factor to predict adequate yields was prior therapy: patients who received a non alkylating agent had an higher mobilization capacity that patients receiving MP (OR 8.7, 95% percent confident interval, 2.2 to 33.4) and a prior iv melphalan therapy reduces the ability to collect an adequate yield rather than receiving MP courses (OR 0.2, 95% percent confident interval, 0.05 to 0.9). Therefore patients were divided in three subgroups: non alkylating agents group (Group I), oral melphalan group (Group II) and intravenous melphalan group (Group III). In the first group we considered 37 patients with median age 57 years (range 34-73): 34 achieved an adequate collection with median progenitors harvested of 10.65 × 10^6/kg. For the second group of 39 patients with median age 62 years (range 35-71), 22 patients mobilized with a median CD34+ collection of 7.95 × 10^6/kg. The third group consisted of 13 patients with median age 59 years (range 49-71), but only 3 patients had an adequate collection with a median of 3.37 × 10^6 CD34+ cells/kg. Among prognostic factors (stage, percentage of bone marrow plasma cells, β2-microglobulin, labelling index, isotype, monoclonal component, Bence Jones proteinuria or maintenance therapy) evaluated at diagnosis there was not an association with progenitor cell yields. All clinical variables which could influence PBPC mobilization were evaluated in multivariate analysis: the specific previous treatment namely the use or not of melphalan, was the only parameter which was significantly correlated with PBPC yields. In conclusion, patients potentially candidates for PBSC transplant, i.e. melphalan therapy should be avoided or PBPC collection performed early in the disease course; for patients who are not considered for transplantation at diagnosis poor performance status or relatively good prognosis, non alkylating therapy (OR 8.7, 95% percent confident interval, 2.2 to 33.4) should be preferred. The use of non-alkylating agents before the PBPC collection is highly recommended and a collection should be performed early in the course of the disease in order to be transplanted at relapse.

WASH OUT OF 99mTc-SESTAMIBI (99mTc-MIBI) IN EVALUATING RESPONSE TO CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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Aim. Wash-out of 99mTc-MIBI is reported to help in predicting response to chemotherapy in various tumors. The aim of this study is to evaluate the role of 99mTc-MIBI in predicting response to chemotherapy in patients with MM. Methods. Forty patients with MM showing diffuse 99mTc-MIBI bone marrow uptake were included in the study. All patients underwent whole body scan at 10 and 60 minutes after tracer injection. Diffuse bone marrow uptake was semiquantitatively scored according to both extension and intensity on the 10 minute scan. Wash-out was computed, after decay correction, as: (10 minutes counts/pixel minus 60 minutes counts/pixel) divided by 10 minutes counts/pixel. All patients were clinically re-evaluated (mean follow-up 26±9 months) after chemotherapy (14 MP, 9 VAD, and 17 VMCP). Results. Of the 40 patients, 10 were considered to be in remission at re-evaluation and 30 showed disease progression. Neither the clinical status at presentation nor the therapeutic regimens were associated with the clinical status at re-evaluation (i.e. remission vs disease progression) (Chi square= 1.8 and Chi square= 1.0, respectively, p=n.s.). The bone marrow uptake score at the 10 minutes baseline 99mTc-MIBI scintigraphy was significantly (p<0.05) higher in patients showing disease progression (3.9±1.4) than in those in remission (2.7±1.4). 99mTc-MIBI wash-out was significantly (p<0.01) higher in patients with disease progression (18.3±9.8) than in those in remission (11.6±6.2). Of the 10 patients showing remission of disease after chemotherapy, the majority (7, 70%) had 99mTc-MIBI wash-out <15% (which was the median for the whole group), while of the 30 patients with disease progression after chemotherapy the majority (20, 67%) had 99mTc-MIBI wash-out >15% (Chi square=4.1, p<0.05). Conclusions. Our preliminary data suggest that in vivo evaluation of 99mTc-MIBI wash-out may help in predicting response to chemotherapy in patients with MM.
ed with HDT: Cyclophosphamide 7 g/m² as mobilizing regimen, autologous peripheral blood transplantation following Melphalan 200 mg/m²: 11 single, 20 double. Four patients received allo-
genic bone marrow transplantation. Overall survival was calcu-
lated from diagnosis to the time of exitus or to the last observa-
tion, with a minimum follow up of 12 months for the survivors. Results. Median overall survival is 31.5 months. The probability
of survival at 60 months is 58.5% for both types of treatment. A
significant difference in favor of HDT is observed only for patients
with advanced stage disease (stage III: 38.9% vs. 50.6% at 60
months; p = 0.04). Conclusions. In our experience, intensive
chemotherapy improved survival in patients at high risk with
advanced stage disease. Conventional chemotherapy can still be
considered a treatment option for the majority of patients with
multiple myeloma. The use of biotherapy can be envisioned and
may improve the prognosis of patients with multiple myeloma.

PO315
HIGH EFFICACY AND SAFETY OF DCEP REGIMEN FOR PERIPHERAL STEM
CELL COLLECTION IN MULTIPLE MYELOMA

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Design and Methods. This study was performed in order to eval-
uate the efficacy of DCEP (dexamethasone 40 mg × 4 d and 4-
day continuous infusion of daily doses of cyclophosphamide 400
mg/m², etoposide 40 mg/m², and cisplatin 10 mg/m² with subse-
quint G-CSF 300 μg s.c. until neutrophil recovery) as mobilizing
therapy and to define its toxicity. Fifty-five MM pts, 30 males
and 25 females, entered the study to receive DCEP followed by G-
CSF as part of a high-dose sequential chemotherapy including
single or double autologous transplantation. The median age was
54 years (range 30-68). Patients staged by Durie and Salmon's
classification were as follows: 36 patients in stage I, 6 in stage II
and 3 in stage III. At the time of mobilization, of the 55 patients,
40 had previously received VAD only, 11 alkylating agents, and 5
radiotherapy. 38 (6%) negative. No false-positive aspirates results were obtained. We observed no complications from bleeding or other morbidi-
ity. No patient refused the offered procedure. The reported data
confirm the high sensitivity (94%) and the predictive value of
PUF. In our hands, it is the diagnostic procedure of choice in
patients with monoclonal gammopathy of undetermined signif-
icance, multiple myeloma and other lymphoproliferative disor-
ders, presenting with symptoms and/or signs suggestive of SA
(restrictive cardiomyopathy, peripheral or autonomic neuropa-
athy, urinary protein loss, coagulopathy, carpal tunnel). The tech-
nique is easy to perform and is virtually devoid of morbidity
complications. It is recommended for patients with risks of bleeding and may obviate more invasive diagnostic testing. In our hands,
PUF resulted useful, reliable, safe, inexpensive, and well tolerat-
ed. However, the examination of the specimen stained with CR
under polarized microscopy requires an experienced, skilful per-
son. We recommend promptly performing the PUF in all patients
with minimal signs or symptoms of suspicious SA since, in
the early diagnosed cases, this may allow the management by
standard and, in selected cases, high dose chemotherapy and auto-
graft, before the occurrence of severe multiorgan damage.
FACTORS ASSOCIATED WITH DISEASE EVOLUTION IN 127 SMOLDERING MULTIPLE MYELOMA PATIENTS

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Smoldering multiple myeloma (MM) is an uncommon form of MM that acts in a biologically similar way to monoclonal gammopathy of undetermined significance. The absence of bone lesions, the low plasma cell labeling index and the stability of the clinical course during a period of many years suggest that patients should be observed without chemotherapy. All cases, however, eventually transform into symptomatic disease. Moreover, the existence of a small SMM subgroup with a high likelihood of evolution to overt MM has been reported. At the present time, patients at high transformation risk are not identified by the available test parameters, and careful monitoring is needed. The existence of predictive factors for disease progression might be essential in selecting patients in whom a very strict monitoring is required. One hundred and twenty-seven consecutive patients with IgG (n=91), IgA (n=31), and double paraprotein (n=5) SMM diagnosed from July 1975 to March 1998 were included in the study. SMM was defined as follows: IgG monoclonal protein >3.5 < 7 g/dL or IgA monoclonal protein > 2 < 5 g/dL, and/or Bence Jones proteinuria > 1 g/24h, and/or bone marrow plasma cells > 10 < 20%, absence of bone lesions, anemia, hypercalcemia, and renal insufficiency. The univariate Cox model was used to identify possible predictors of malignant evolution. At a median follow-up of 72 (range, 12 to 247) months, 25 SMM (19.7%) evolved to overt MM. At univariate analysis, variables associated with an increased probability of evolution were: the IgA isotype, detectable Bence Jones proteinuria and > 10% bone marrow plasma cell levels. Age, sex, serum P-/microglobulin, serum albumin, erythrocyte sedimentation rate, the presence of double monoclonal component, and the reduction of one or two serum polyclonal immunoglobulins were not associated with disease progression. The prevalence of evolution of SMM patients is higher than that reported for monoclonal gammopathy of undetermined significance. Although further investigations and longer follow-up studies are necessary to draw definitive conclusions on SMM prognosis, our study suggests that the evaluation of paraprotein isotype, urinary paraprotein and bone marrow plasmacytosis might help to identify at presentation patients with SMM requiring a very strict monitoring.

PAMIDRONATE TREATMENT IN PATIENTS WITH STAGE I MULTIPLE MYELOMA: PRELIMINARY RESULTS OF A CLINICAL PERSPECTIVE MULTICENTER STUDY

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Stage I multiple myeloma (Durie-Salmon) is not usually treated, unless signs of progression appear. Nevertheless, the potential evolution involves approximately 20-30% of patients, whose identification at diagnosis is not possible. Disodium pamidronate shows direct or indirect antiproliferative effect on myeloma cells. Starting from these considerations, we undertook a clinical prospective study in order to verify pamidronate antiproliferative action in stage I myeloma. Eligible patients were allocated to two groups: the experimental group received pamidronate (Aredia, Novartis) at a dose of 60 mg by intravenous infusion every 28 days for at least six months, the control group was not treated. Until March 2001 we have enrolled 12 cases, 6 in the treated group and 6 in the control group. Characteristics of patients: 3 males, 9 females, mean age 72 years (range 54-88). 10 IgG, 2 IgA. After six months of treatment or observation, we obtained the following results. Treated: mean variation of monoclonal component (MC) +2.41% (range –6.7%-+22.80%), SD 10.48, median variation -0.78%, 1 progression, no new skeletal event. Treatment was well tolerated: only 1 case of asymptomatic and transient hypocalcemia. Not treated: mean variation of MC +9.85% (range -4.5%-+2.41%), SD 8.26, median variation +10.05%, no progression, new skeletal event in 1 patient. Although MC increase was greater in the control group, our analysis on these preliminary data does not show statistically significant differences. The aim of this study is the enrollment of at least 100 patients with a prolonged follow-up, to verify possibly significant results.

COMBINED PAMIDRONATE AND DEXAMETHASONE THERAPY AS MAINTENANCE IN MULTIPLE MYELOMA RESPONDER PATIENTS: EXPERIENCE OF A SINGLE CENTER

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The best approach for MM patients, responders to induction therapy, remains a still debated issue. It is now well established that pamidronate decreases the progression of osteolytic lesion, bone pain and fractures also in MM patients. In vitro studies demonstrated that this biphosphonates can inhibit myeloma cell proliferation inducing apoptosis (Shipman et al., 1997, Aparicio et al.1998), and may have a direct effect on the tumor cells giving an increase in patients survival (Berenson et al., 1998). Based on these observations and on the efficacy of dexamethasone (dexa), considered an active agent for MM patients, we decided to evaluate the efficacy and safety of the association
pamidronate and dexamethasone in maintaining hematological and clinical response after induction therapy. From July 1998 to February 2001, among 73 MM patients followed at our Unit, 14 (10 M/4 F, median age 69 years, range 52-83 years) newly diagnosed cases (9 IgG, 2 IgA and 3 light chain) with a median follow-up of 6 months, entered in this study. At diagnosis 4 patients were classified as having stage IA, 9 IIa and 1 IIb; as induction, all patients but one received melphalan plus dexamethasone (DEXA regimen) followed by autologous peripheral blood stem cells transplant. After induction all patients were considered responders (>50% decrease of monoclonal component, Hb level >10g/dL, reduction in bone pain, improvement in Performance Status). Maintenance therapy consists of at least 6 courses of Pamidronate (90 mg t.d.) combined with Dexamethasone (20 mg t.d.) given for 28 days. All patients received this treatment on an out-patient basis, during maintenance hematological, biochemical and clinical controls were performed monthly. The median follow-up of the 14 patients entered in this study is 11 months (range 6-30 months), median number of maintenance courses given was 11 (range 6-24). To date 13 patients are alive, of these 12 patients persist in continuous response, 1 patient relapsed after 24 months and only 1 patient died because of infection in relapse of disease. Median duration of response was 11 months (range 6-24), median overall survival was 11 months (range 6-30). In our experience this combined treatment demonstrated to be effective in maintaining durable hematological and clinical response; in older patients mainly, therapy was well tolerated improving the quality of life. These preliminary, promising results need to be confirmed in a larger cohort of patients with a longer follow-up.

**PO320**

**EPIDEMIOLOGIC ASPECTS OF MULTIPLE MYELOMA IN SARDINIA, 1974-1993**

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We have collected all cases of hematological malignancies (HM) newly diagnosed in the resident population of Sardinia in the period 1.1.1974-31.12.1993. Cases were searched through a personally done examination of all registers of all medical departments and of all pathology institutions in that time operating in Sardinia; confirmation of diagnosis, date of diagnosis and demographic data of patients were obtained through a personally done consultation of clinical records. Epidemiologic evaluations have been done according to the recommendations of dos Santos Silva. A total of 7339 cases of HLM were found. Of them, 824 had a diagnosis of multiple myeloma (MM), representing 11.3% of all cases of HM. The first cases (only three in 20 years) were observed in age group 25-34. Number of cases presenting 11.3% of all cases of HM. The first cases (only three in 20 years) were observed in age group 25-34. Number of cases presenting 11.3% of all cases of HM in stage II or III (Chi square 63.6, p<0.0001). At clinical re-evaluation all patients were considered responders (>50% decrease of monoclonal component, Hb level >10g/dL, reduction in bone pain, improvement in Performance Status). Maintenance therapy consists of at least 6 courses of Pamidronate (90 mg t.d.) combined with Dexamethasone (20 mg t.d.) given for 28 days. All patients received this treatment on an out-patient basis, during maintenance hematological, biochemical and clinical controls were performed monthly. The median follow-up of the 14 patients entered in this study is 11 months (range 6-30 months), median number of maintenance courses given was 11 (range 6-24). To date 13 patients are alive, of these 12 patients persist in continuous response, 1 patient relapsed after 24 months and only 1 patient died because of infection in relapse of disease. Median duration of response was 11 months (range 6-24), median overall survival was 11 months (range 6-30). In our experience this combined treatment demonstrated to be effective in maintaining durable hematological and clinical response; in older patients mainly, therapy was well tolerated improving the quality of life. These preliminary, promising results need to be confirmed in a larger cohort of patients with a longer follow-up.

**PO321**

**PREDICTIVE VALUE OF 99mTc-SESTAMIBI (99mTc-MIBI) IN PATIENTS WITH MULTIPLE MYELOMA**

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**Aim.** 99mTc-MIBI scintigrapy proved to be useful in evaluating patients with MM. The aim of this study is to evaluate its predictive role in patients with MM. Methods: One hundred and eighteen patients constituted the study group. All patients underwent a baseline whole body 99mTc-MIBI scintigraphy (10 minutes after i.v. tracer injection) and were clinically re-evaluated at a mean follow-up of 19.4+9 months. The scans were classified as N (physiological uptake), D (diffuse marrow uptake), F (focal uptake), and D+F. The intensity of diffuse bone marrow uptake was semiquantitatively scored. Results. At baseline 99mTc-MIBI, of the 51 patients with pattern N only 8 (16%) were in stage II or III, of the 38 with pattern D 13 (34%) were in stage II or III, of 29 with pattern F or D+F 27 (93%) were in stage II or III (Chi square 63.6, p<0.0001). At clinical re-evaluation, 20 patients were in remission, 49 were in stable condition, 32 had disease progression, and 17 had died. Nine of the 51 (17%) patients with baseline pattern N had progression of disease, 23 (61%) of the 38 patients with pattern D had disease progression, and 17 (59%) of the 29 with pattern F or D+F had progression. This increase is not due solely to ageing of Sardinian population: indeed it is evident also in the curve of age-standardized annual incidence rates (standard: Sardinian population at 1981 census). We must consider the possibility of a real increase of incidence of MM and/or that of an increase of diagnosis in recent years of previously undetected patients (due to increased physician awareness of the disease and more available clinical and laboratory facilities, especially in the elderly people). Age standardized incidence rate (standard: world population) in Sardinia,1974-1993 and in other populations is set out in the following table:

<table>
<thead>
<tr>
<th>Age adjusted incidence rates (standard world population)</th>
<th>Cases × 100,000 × year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardinia, 1974-1993</td>
<td>1.99 1.73</td>
</tr>
<tr>
<td>Italy, 1980</td>
<td>2.41 1.79</td>
</tr>
<tr>
<td>France, 1990</td>
<td>2.54 1.93</td>
</tr>
<tr>
<td>France-Cote d’Or, 1980-1996</td>
<td>3.0 2.3</td>
</tr>
<tr>
<td>Sweden, 1990</td>
<td>4.01 2.56</td>
</tr>
<tr>
<td>Germany, 1990</td>
<td>2.76 1.96</td>
</tr>
<tr>
<td>USA, 1990</td>
<td>4.13 2.84</td>
</tr>
</tbody>
</table>

Thalidomide is a glutamic acid derivative with anti-angiogenic and immunomodulating properties, that has recently demonstrated to be active in relapsed/refractory multiple myeloma (MM) patients. Several in vitro studies have shown a synergistic effect of the combination thalidomide plus dexamethasone in MM cell lines, a finding confirmed by preliminary clinical data. These observations provided the rationale of a phase II clinical trial of thalidomide+dexamethasone which was started at our Institute for the treatment of advanced relapsed/refractory MM patients. From December 2000 to May 2001, 18 patients (15 M/3 F) were enrolled in the trial; their median age was 61 years, all patients were in stage III, median β2-microglobulin was 5.1 mg/L, median bone marrow plasma cell infiltration was 70%. Eleven patients had previously received stem cell transplantation, either autologous (2 patients) or autologous (single = 5 patients, double = 4 patients). Seven patients had been previously treated with thalidomide as a single agent, 4 of them were refractory, while 3 showed disease progression following an initial response. Thalidomide was firstly administered at 100 mg/day; if well tolerated, the dose was increased serially by 200 mg/day every other week to a maximum of 600 mg/day. Dexamethasone was administered at 40mg/day for 4 consecutive days, at three week intervals. Median administered dose of thalidomide was 300 mg/day, WHO grade > II toxic effects were constipation (43%), lethargy (37%) and peripheral neuropathy (12%). At present, 14 patients are evaluable for response, 3 (21%) showed a 50% reduction in serum or urine M protein concentration and 7 (50%) showed > 25% response. Interestingly, a response was noted in 4/6 evaluable patients who were refractory to, or relapsing after, previous treatment with thalidomide alone. After 3 months median follow-up, 9/10 patients are alive and progression-free, and one patient has relapsed. These preliminary data seem to suggest that the combination thalidomide+dexamethasone for the management of advanced MM 1) increases response rates in comparison to historical series of patients treated with thalidomide as single agent (see Tosi et al, current issue); 2) allows rescue of a certain fraction of patients refractory to thalidomide alone. Based on these results, which need confirmation in larger series of patients, thalidomide and dexamethasone holds promise to be one of the most active salvage therapies presently available for patients with advanced relapsed/refractory MM who are not eligible for high-dose treatment programs.

PO323
THALIDOMIDE PLUS DEXAMETHASONE IS AN EFFECTIVE SALVAGE THERAPY FOR PATIENTS WITH ADVANCED RELAPSED AND REFRAC'TORY MULTIPLE MYELOMA

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In MM cell lines, a finding confirmed by preliminary clinical data. These observations provided the rationale of a phase II clinical trial of thalidomide+dexamethasone which was started at our Institute for the treatment of advanced relapsed/refractory MM patients. From December 2000 to May 2001, 18 patients (15 M/3 F) were enrolled in the trial; their median age was 61 years, all patients were in stage III, median β2-microglobulin was 5.1 mg/L, median bone marrow plasma cell infiltration was 70%. Eleven patients had previously received stem cell transplantation, either autologous (2 patients) or autologous (single = 5 patients, double = 4 patients). Seven patients had been previously treated with thalidomide as a single agent, 4 of them were refractory, while 3 showed disease progression following an initial response. Thalidomide was firstly administered at 100 mg/day; if well tolerated, the dose was increased serially by 200 mg/day every other week to a maximum of 600 mg/day. Dexamethasone was administered at 40mg/day for 4 consecutive days, at three week intervals. Median administered dose of thalidomide was 300 mg/day, WHO grade > II toxic effects were constipation (43%), lethargy (37%) and peripheral neuropathy (12%). At present, 14 patients are evaluable for response, 3 (21%) showed a 50% reduction in serum or urine M protein concentration and 7 (50%) showed > 25% response. Interestingly, a response was noted in 4/6 evaluable patients who were refractory to, or relapsing after, previous treatment with thalidomide alone. After 3 months median follow-up, 9/10 patients are alive and progression-free, and one patient has relapsed. These preliminary data seem to suggest that the combination thalidomide+dexamethasone for the management of advanced MM 1) increases response rates in comparison to historical series of patients treated with thalidomide as single agent (see Tosi et al, current issue); 2) allows rescue of a certain fraction of patients refractory to thalidomide alone. Based on these results, which need confirmation in larger series of patients, thalidomide and dexamethasone holds promise to be one of the most active salvage therapies presently available for patients with advanced relapsed/refractory MM who are not eligible for high-dose treatment programs.

PO323
HEPATITIS C VIRUS INFECTION AND MULTIPLE MYELOMA

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In the last few years hepatitis-C virus (HCV) has been implicated in the pathogenesis of diverse processes originating from B-clonal lymphoid proliferation such as mixed cryoglobulinemia (MC), Waldenström’s macroglobulinemia (WM) and B-cell non-Hodgkin’s lymphomas (NHL). Moreover, HCV infection has been found to be highly prevalent in subjects with chronic liver disease and strongly associated with hepatocellular carcinoma; it has been postulated that the virus favors the onset of lymphoproliferative disorders and other tumors correlated with immune disorders. The aim of this work was to investigate the prevalence of hepatitis-C infection in 98 consecutive patients (mean age, 69±11.2) suffering from multiple myeloma (48 women and 50 men) who were hospitalized in our hospital from January 1998 to April 2001. Patients without a history of cancer or autoimmune disease and hospitalized in the same period in our hospital were used as control. All subjects investigated were HIV negative. Antibodies to HCV were detected with a third-generation ELISA test (Ortho Diagnostic System, Raritan, NJ, USA); positive samples were tested in duplicate and, if reactive, confirmed by a third generation RIBA test (RIBA 3 Chiron Corporation, Emeryville, CA, USA). In contrast to previous reports showing a higher prevalence of HCV infection in multiple myeloma than in the normal population, our results showed a prevalence of HCV infection of 5.2% in patients with multiple myeloma compared with a prevalence of 5.4% in the controls: the prevalence of HCV infection in male multiple myeloma patients was 3.1% and in female patients 2.1%. At present, in some series such as ours, there is insufficient evidence to conclude that chronic HCV infection increases the risk of the development of multiple myeloma, while in others it has been suggested that HCV may play a direct pathogenetic role in some lymphoproliferative disorders. The relationship between HCV and multiple myeloma and the oncogenic role of HCV remains uncertain. The role of HCV infection in the pathogenesis of multiple myeloma needs to be confirmed by extensive epidemiologic studies.

PO324
PREVALENCE OF HEPATITIS C VIRUS ANTIBODIES IN PATIENTS WITH MONOCLONAL GAMMOPATHY

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Hepatitis C virus (HCV) infection is responsible for both liver diseases, i.e. chronic hepatitis, cirrhosis and hepatocellular car-
cinobulinemia, and several extrahepatic disorders including mixed cryoglobulinemia, membranoproliferative glomerulonephritis, porphyria cutanea tarda, B-cell non-Hodgkin's lymphoma and monoclonal gammopathy. While there is a clear evidence for an association between HCV infection and most of these diseases, a link between HCV and monoclonal gammopathy is still controversial. These data and the relatively high rate of HCV infection observed in subjects with monoclonal gammopathy, prompted us to investigate retrospectively the prevalence of HCV antibodies among 87 patients (34 men and 53 women) suffering from various monoclonal gammopathies who were admitted to our Hema-
tology Department in the last years. The population (mean age, 60.06±11.73 years) included: multiple myeloma (32), mono-
clonal gammopathy of uncertain significance (44), Walden-
ström's macroglobulinemia (3), non-secretory myeloma (2), light-chain myeloma (5) and bicalonal myeloma (1). A group of 126 subjects (32 men and 94 women; mean age, 55.03±14.88 years) who had no history of lymphoproliferative disorders or autoimmune diseases were used as controls. The serologic diagnosis of HCV was investigated by second or third generation ELSA test; reactive sera were also tested by a recombinant immunoblot assay (RIBA). The association between monoclonal gammopathy and HCV was assessed by a chi-square test. Our results showed a prevalence of HCV-antibodies in 21 patients (24.13%) with monoclonal gammopathy and in 16 subjects (12.70%) of the control group and this difference resulted signif-
ificantive (p<0.05). The prevalence of HCV-antibodies in males with monoclonal gammopathy was 32.35% and in females 18.86%, while the percentage in the control group was respec-
tively 18.75% and 10.64%. In our opinion, these data, even if pre-
liminary and incomplete, are interesting and may suggest that HCV could play a role also in the pathogenesis of mono-
clonal gammopathy, as already hypothesized by some authors. However, our data must be better studied by detecting the HCV-
RNA with a polymerase chain reaction in subjects resulted pos-
itive to HCV antibodies. Although lymphotropism is a well-rec-
ognized feature of HCV, its exact role in development of extra-
hepatic disorders is mostly unknown at the moment; but inter-
ingest explanation is offered by the recent identification of the interaction between HCV envelope protein E2 and CD81 on both hepatoocytes and lymphocytes and the finding of an elevated fre-
quency of proto-oncogene bcl-2 recombination with corre-
sponding overexpression of bcl-2 anti-apoptotic oncoprotein in peripheral monocellular cells of HCV infected subjects. In this light and since HCV is an important health problem throughout southern Italy, we believe that further studies are needed to investigate the real impact of HCV in development of mono-
clonal gammopathy.

**PO325**

**USE OF THALIDOMIDE IN REFRACTORY/RELAPSED MULTIPLE MYELOMA: A MONOINSTITUTIONAL EXPERIENCE**

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After the first experience of the Little Rock group (N Engl J Med 1999; 341:1565-71) thalidomide has been used in a large number of hematologic institutions. We tested the drug in a small selected pre-treated populations. Cases. We treated 11 refractory/relapsed MM patients (at least two lines of therapy), 4 M and 7 F, 56-81 years old 6 MM IgG (κ), 2 MM IgG (λ), 1 MM IgA (κ) ed 1 MM IgA (λ). The schedule of treatment was the fol-
lowing: 100 mg daily during the first two weeks, then a dose escalation of 100 mg every two weeks to the maximum tolerat-
ed dose. Then when the response was reached thalidomide was reduced to the lower effective dose. Two patients are not valu-
able for response because too short time of treatment; two pts stopped thal for side effects, 1 for cramps and 1 for hyperpyrexia. Somnolence was present in all patients and was dose limiting. Results. Values of monoclonal component, bone marrow plas-
mocytosis, schedule of treatment, type of response and survival are reported in the table below.

<table>
<thead>
<tr>
<th>#</th>
<th>M.C. value</th>
<th>I.G. value</th>
<th>B.M. PL</th>
<th>Max dosage</th>
<th>Mant Dosage</th>
<th>Response</th>
<th>Survival</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1</td>
<td>4010</td>
<td>75</td>
<td>200</td>
<td>200</td>
<td>Progress</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
<td>2290</td>
<td>60</td>
<td>200</td>
<td>50</td>
<td>PR (50%)</td>
<td>10+</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>2940</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>Progress</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>2.7</td>
<td>1870</td>
<td>40</td>
<td>600</td>
<td>200</td>
<td>RP (50%)</td>
<td>9+ (in RP)</td>
</tr>
<tr>
<td>5</td>
<td>4.1</td>
<td>2760</td>
<td>50</td>
<td>200</td>
<td>100</td>
<td>RP (50%)</td>
<td>9+ (in RP)</td>
</tr>
<tr>
<td>6</td>
<td>3.4</td>
<td>4140</td>
<td>12</td>
<td>400</td>
<td>200</td>
<td>RC (&gt;95%)</td>
<td>8+ (in RC)</td>
</tr>
<tr>
<td>7</td>
<td>2.8</td>
<td>2790</td>
<td>80</td>
<td>100</td>
<td>-</td>
<td>Progress</td>
<td>3+ (in P)</td>
</tr>
<tr>
<td>8</td>
<td>n.v.</td>
<td>n.v.</td>
<td>20</td>
<td>200</td>
<td>100</td>
<td>RP (Midol)</td>
<td>6+ (in RP)</td>
</tr>
<tr>
<td>9</td>
<td>3.9</td>
<td>4150</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>n.v.</td>
<td>8</td>
</tr>
</tbody>
</table>

Conclusions. Thalidomide has been active in 5/7 pts evaluable for response (4 PR; 1 CR) with a duration of response of 6-10+ months. Although in some cases we observed somnolence the drug seems active in MM patients and we are evaluating timing of thalidomide therapy in our MM treatment strategy.

**PO326**

**SALVAGE TREATMENT WITH THALIDOMIDE IN REFRACTORY MULTIPLE MYELOMA PATIENTS**

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Thalidomide (Thal) is an immunomodulating agent which was suggested to be effective in hematological malignancies because of an inhibitory effect on growth and survival of tumor and blood marrow stromal cells. Moreover, recent small clinical trials demonstrated a clinical activity against refractory myeloma. In our Center 18 previously treated patients with refractory or relapsing MM received oral Thal as a single agent for a median of 110 days (range 7-270). Median patients age was 60 years (range 37-71), median time from diagnosis was 48 months (range 9-155); all patients had previously received 2 to 6 (medi-
ian) 3 regimens of chemotherapy and 8 had undergone one (4 patients) or two (4 patients) courses of high-dose treatment with autologous (7 patients) or allogeneic (1 patient) stem cell support. The starting Thal dose was 100 mg daily and the dose was increased by 200 mg every two weeks. Four patients stopped assuming 100 mg daily of Thal during the first two weeks of treatment because of an adverse event (2 renal failure due to myeloma progression, one sudden death during dialysis, one
Angiogenesis was observed in active MM, thalidomide was used to have an antiangiogenetic effect. On the basis that bone marrow survival curve has not been observed even after high dose chemotherapy, a survival for patients with MM is about 3 years and a plateau of least 400 mg seems to be necessary to have clinical activity, but conventional-dose than high-dose therapy. A daily dose of al thalidomide, 9 patients had progressive disease and one patient was in PR after one course of high-dose chemotherapy. Median overall survival was 241 days and seven patients are still alive, median overall survival was not reached. Conclusion: Even if our patient population is small we confirm that thalidomide is an active drug in the management of resistant MM patients. The overall response and the quality of life of treated patients are quite encouraging, considering the toxicity and the efficacy of standard therapy in resistent disease. The demonstrated activity of thalidomide associated with the low toxicity profile emphasizes its possible combination with chemotherapy as salvage regiment as induction treatment. It is not yet clear was the best dosing and the optimal timing of administration. In our institute, we are exploring the possibility of administering thalidomide as a maintenance treatment after high dose chemotherapy in patients not in CR.

Multiple myeloma (MM) is an incurable disease; the median survival for patients with MM is about 3 years and a plateau of survival curve has not been observed even after high dose chemotherapy. There are no effective treatments for relapsed patients. For these reason, new therapeutic approaches are needed. Thalidomide, a nonbarbiturate sedative used during preg-
nancy, with an antiangiogenetic effect. On the basis that bone marrow angiogenesis was observed in active MM, thalidomide was used in these patients. Patients and methods. From October 1999 to January 2001, 10 patients with MM were treated with thalidomide given orally by single evening dosing of 100 mg/d for 2 weeks, and then increased by 100 mg/day every 2 weeks, up to an intentional maximal dose of 800 mg/d. The median age was 57 years (range 45-79), 5 were males and 5 females. At the start of thalidomide, 9 patients had progressive disease and one patient was in PR after one course of high-dose chemotherapy. All patients were heavily pre-treated with more than two chemotherapy regimens. In 6 patients one or more courses of high dose chemotherapy were performed (3 courses in one patient, 2 courses in two patients, 1 course in three patients) and 5 patients were also treated with local radiotherapy for bone disease. One aged patient (79 years) was resistant to first-line melphalan and prednisone alone. Performance status was poor (<70%) in all patients. Patient pre-treatment evaluation included complete blood counts, renal and liver function, serum and urinary electrophoresis and immunoelectrophoresis and immunoglobulins, β2microglobulin, C-reactive protein (CRP), LDH serum level; bone marrow analysis was performed to determine percentage of plasmacells and chromosomal analysis. All patients were monitored for serologic response and toxicity every two weeks for two months, and thereafter every four weeks. During follow-up bone marrow analysis was performed in case of serologic complete response. Results. All patients were evaluable for response. Median daily dose of thalidomide was 280 mg. No patients tolerated more than 500 mg/day. Five patients (50%) showed a paraprotein level decline after one month of therapy. After 8 months of median follow up of we obtained 1 CR (10%), 3 PR (30%), 2 MR (10%), 2 SD (20%), 1 progressive disease (10%). A patient in PR after one course of high dose chemotherapy obtained serologic and morphologic CR after 3 months of thalidomide. Response was durable (>5 months) in 66% of cases. One patient with a minimal response after one month of therapy was lost to the follow up. All patients improved performance status and reduced analgesic drugs. Treatment was well tolerated without grade III/IV toxicity: no patients discontinued thalidomide for toxicity. In three patients a grade II neutropenia was registered and one patient was hospitalized for infection (pneumonia). One patient complained of morning orthostatic hypotension. No patients developed a cutaneous rash. Median follow up was 241 days and seven patients are still alive, 5 on treatment with thalidomide. Median overall survival was not reached. Conclusion: Even if our patient population is small we confirm that thalidomide is an active drug in the management of resistant MM patients. The overall response and the quality of life of treated patients are quite encouraging, considering the toxicity and the efficacy of standard therapy in resistent disease. The demonstrated activity of thalidomide associated with the low toxicity profile emphasizes its possible combination with chemotherapy as salvage regiment as induction treatment. It is not yet clear was the best dosing and the optimal timing of administration. In our institute, we are exploring the possibility of administering thalidomide as a maintenance treatment after high dose chemotherapy in patients not in CR.
main part of hemopoietic cells. Moreover, physical parameters can give some help in the identification only at onset, because the bone marrow infiltration is higher, but not in the apheresis products where the plasma cell quantitation is more important but the infiltrate is lower. A good cytofluorimetric detection of plasma cells without misunderstandings with other leukocytes populations can be obtained with a double staining with the monoclonal antibodies CD38bright/CD45dim. More recently, to detect plasma cells, a monoclonal antibody (BB4 CD138) can be used that binds a molecule expressed at high levels in plasma cells and involved in the cellular adhesion to collagen (Syndecan-1). This antibody allows the detection of plasma cells with results similar to those obtained with double staining with CD38/CD45. The use of only one antibody to detect plasma cells gives us the chance to study by double or triple staining other plasma cell characteristics, i.e. the expression of the multidrug resistance related proteins (PGP, MRP, LRP) or of the apoptosis related proteins (Bcl2, FAS, Annexin V). Moreover, the immunomagnetic monoclonal antibody CD138 (MiniMacs, Miltenyi) allows a positive separation of plasma cells. In this study we evaluated the fluorescence intensity of ICD138 on normal peripheral blood and bone marrow plasma cells, in multiple myelomas (MM) at onset and after induction therapy (VAD) and in peripheral blood stem cell collections. The following table reports the results obtained.

<table>
<thead>
<tr>
<th>CD138 MFI</th>
<th>normal PB</th>
<th>normal BM</th>
<th>MM at onset</th>
<th>MM post VAD</th>
<th>PBSC</th>
</tr>
</thead>
<tbody>
<tr>
<td>20±6</td>
<td>32±5</td>
<td>51±19</td>
<td>24±20</td>
<td>25±3</td>
<td></td>
</tr>
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</table>

In MM at onset CD138 seems to have a higher fluorescence intensity but because of the small number of the cases no statistical analysis was performed.

P0329
DEVELOPMENT OF HODGKIN’S DISEASE FIVE YEARS AFTER AUTOLOGOUS STEM CELLS TRANSPANTATION FOR MULTIPLE MYELOMA

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We report the case of a 53-year old man who developed Hodgkin’s disease five years after autologous peripheral blood stem cell transplantation (PBSCT) for multiple myeloma (MM). In July 1994 the patient was submitted to PBSCT for MM (IgG, λ) after six courses of VAD induction regimen, which obtained good response. At the time of transplant bone marrow infiltration was 2% of plasma cells. In May 1993 bone marrow biopsy showed a diffuse infiltration up 70% of rather atypical plasma cells. No Bence Jones or renal impairment was demonstrated. Monoclonal component was hardly detectable. No skeletal lesion presented. He did not show other laboratory abnormalities. PBSCT was performed under conditioning regimens of melphalan at the dose of 250 mg/m² (day -3) and total body irradiation (total dose 800 cGy day -1). During the months following transplant he was well with disappearance MM in his marrow but mild M-component was still evident. Interferon-α was started as maintenance therapy in January 1995. Since 1995 to 98 he suffered from frequent acute, feverish, bacterial bronchitis and pneumonia episode. In March 1998 the patient was hospitalized in the heart coronary unit because of acute myocardial infarction. Then interferon-α was stopped. In July 1999 normochromic normocytic anemia was detected and chest X ray revealed mediastinal lymph node enlargement. He was submitted to computed tomography with abdominal and mediastinal nodes enlargement results. Bone marrow biopsy showed coexistence of infiltration of plasma cells (20%) plus Hodgkin’s cells (20%). Fine needle aspiration of the lymphatic mass was performed without demonstration of pathological cells. The patient’s clinical course was getting worse and the liver dysfunction became evident with conjugate hyperbilirubinemia jaundice so that chemotherapy could not be administered. The patient died two months later for pulmonary oedema and liver failure. The post-mortem examination revealed HD spread to marrow, spleen, liver, retroperitoneal, cellular, and mediastinal nodes. Multiple myeloma in the marrow and renal, lung and hepatic amyloidosis. Bone marrow recipient are at an increased risk of later malignancy. Usually post-BMT malignant neoplasm includes B-cells limophoproliferative disorders, myelodysplastic syndromes, acute myelogenous leukaemia, non-Hodgkin’s lymphoma and rarely Hodgkin’s disease. Increased incidence of HD has been reported after allogeneic bone marrow transplantation, probably associated with viral infection (EBV, HIV1) that alter the human immune system. MM and HD have the same B-cells lymphoid origin. Also show the same karyotypic abnormalities. Again IL-6 is the central tumour growth factor for myeloma cells. High levels of IL-6 was found in HD patient with advanced disease. Those high levels of IL-6 in the marrow microenvironment might directly stimulate the plasma cells there. We suppose that post-autologous bone marrow transplant severe immune dysfunction of our patient (several bacterial and viral episode) support HD onset. Then through IL-6 HD marrow cells might stimulate remaining long living plasma cells so cause the MM relapse and amyloidosis. Documented cases of simultaneous occurrence of MM and HD has occasionally been reported (Five cases have been described). To the best of our knowledge this is the second report about developed HD after autologous peripheral stem cell transplantation for multiple myeloma. One noteworthy feature of this report is the long latency (five years after transplant) of the disease development after PBSCT.
Thalidomide combined with chemotherapy for relapsed multiple myeloma may be associated with increased risk of thrombo-embolic events

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Introduction. The role of thalidomide (TDM) is emerging in the treatment of relapsed or resistant multiple myeloma (MM); several recent reports have highlighted its efficacy in this disease: about 1/3 of the cases show a response with TDM given as a single agent or in combination with dexamethasone ± chemotherapy (CT), but only few achieve a CR. Adverse effects are mainly related to acquired factors, tests for hereditary thrombophilia may be performed in this patient but none of these had mentioned the presence of Gaucher cells. Maybe they have been mistaken for plasmacells. However, the morphological features of the two kinds of cells are quite different and easily recognizable by an expert hematologist.

Ash Meeting 2000 by Kropff et al. 3 We report on 3 further cases of thrombo-embolic events (TEE) in relapsed MM treated with TDM combined with chemotherapy (TDM/CT). Patients and methods. Some of the patients’ details are described below in Table 1. None of the patients had previous DVTs and only patient 1 had acquired predisposing factors to thrombosis. The timing, site of DVT, disease condition and TDM dose at the time of diagnosis of DVT are reported in Table 2.

Conclusions. Recent reports of TEE and TMD treatment deal with patients with SLE with thrombotic risk most likely due to the presence of anti-CL/PL antibodies. 1 In larger studies of TMD in MM as a single agent 2 no mention is made of TEE. Kropff’s 3 and our patients developed DVT while on TMD/CT; in our cases TEE occurred at the time of bone marrow recovery but none of them occurred in elderly patients, but in cases described in the literature the diagnosis of MM has been made simultaneously or subsequently to the GD diagnosis. In our case GD was diagnosed ten years later. During the long history of disease, several bone marrow evaluations had been performed in this patient, but none of these had mentioned the presence of Gaucher cells. Maybe they have been mistaken for plasmacells. However, the morphological features of the two kinds of cells are quite different and easily recognizable by an expert hematologist.

References


PO332
GEMCITABINE AND GEMCITABINE-CISPLATIN IN RELAPSED-REFRACTORY MULTIPLE MYELOMA: FINAL ANALYSIS

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In pursuit of new drugs to treat relapsed/refractory multiple myeloma (MM), we considered gemcitabine which exerts antitumor activity against many refractory malignancies, administered...
alone or in combination with cisplatin. Sixteen patients with advanced MM (3 resistant, 13 relapsed) received intravenous gemcitabine as a single agent at a dose of 1250 mg/m², over 30 minutes infusion, on day 1, 8, 15 and cisplatin 70-80 mg/m² on day 1, 8, 15 and cisplatin 70-80 mg/m² on day 1 every 28 days. Median age of patients was 68 years (range 57-78) and they had previously received a median of 3 chemotherapy regimens (range 1-4; two of them received high-dose therapy). Stable disease (SD), minor response (MR), partial response (PR) and complete response (CR) were defined as < 25%, 25-50%, 50-75% and > 75% decrease of the paraprotein level, respectively, without increase of the bone marrow plasmacells and/or of bone lesions. No grade 4 toxicity was seen after 147 gemcitabine infusions, whereas 3 grade neutropenia and thrombocytopenia were seen in 21.2% and 13.3% of the 90 gemcitabine - cisplatin infusions, respectively. Nausea-vomiting and organ system toxicity were negligible for both the regimens. After 3 courses of gemcitabine alone, 5 patients (31.2%) had a response (3 MR, 1 PR, 1 CR), 8 (50%) stable disease and 3 (18.8%) disease progression. Ten patients received gemcitabine - cisplatin: 2 progressed, 4 maintained stable disease whereas 4 patients, unresponsive to gemcitabine, obtained a response (1 MR, 3 PR). With a median follow-up of 13 months (range 8-17.5), 7 patients (43.7%) died; 5 (31.2%) have disease progression, 1 (6.25%) relapsed, 1 is still in PR (+11 months) and 2 (12.5%) have stable disease. Median time to treatment failure (TTF) was 8 months (CI 95%: 7.6-8.4) and median overall survival (OS) was 16 months (CI95%: 10-22). These results show that gemcitabine and gemcitabine -cisplatin are well tolerated although the latter regimen can be deeply myelosuppressive. The advantage of outpatient management without severe side-effects and thus with an improvement of the quality of life.

1 (9%) obtained a complete remission, 2 (18%) a partial remission, 8 (72%) a stable disease; and only one of them progressed after six month of therapy. Median follow-up was 200 days (range 80-450). Median time to achieve the best response was 60 days (range 30-190). Side effects included grade 2 constipation in 4 pts (36%), grade 2 skin reactions in 1 patient (9%), grade 1-2 neurotoxicity in 6 pts (54%), and a deep venous thrombosis in 1 pt (9%). We compared these results retrospectively with those observed in a similar cohort of ten refractory MM pts treated with chemotherapy according to the scheme CAV (CCNU, melphalan, etoposide). Data relative to the therapy-related toxicity in the two groups are reported in the table below:

<table>
<thead>
<tr>
<th>No. patients with:</th>
<th>Thalidomide</th>
<th>CAV</th>
</tr>
</thead>
<tbody>
<tr>
<td>need of hospitalisation</td>
<td>3/11 (27%)</td>
<td>7/10 (70%)</td>
</tr>
<tr>
<td>grade 3-4 infections</td>
<td>2/11 (18%)</td>
<td>3/10 (30%)</td>
</tr>
<tr>
<td>transfusion requirement</td>
<td>1/11 (9%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>grade 3-4 extra-hematological toxicity</td>
<td>0/11 (0%)</td>
<td>0/10 (0%)</td>
</tr>
<tr>
<td>therapy-related grade IV neutropenia</td>
<td>0/11 (0%)</td>
<td>5/10 (50%)</td>
</tr>
</tbody>
</table>

Interpretation and Conclusions. Low dose thalidomide is an effective therapy for refractory-relapsed MM and the majority of patients show a good compliance. Comparison with the chemotherapy group demonstrates that thalidomide also gives the advantage of an outpatient management without severe side-effects and thus with an improvement of the quality of life.

P0334 MENINGEAL AND CEREBRAL INVOLVEMENT IN MULTIPLE MYELOMA PATIENTS: REPORT OF 4 CASES

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Cerebral involvement is an unusual complication in multiple myeloma (MM); in most of the patients reported in the literature it was a terminal event in a progressive disease with high tumor mass and circulating plasma cells (PC). Recently we followed at our Division 4 MM patients who presented myelomatous meningsitis with multiple intraparenchymal lesions or a localized cerebral plasmacytoma. Two of these patients relapsed with meningeal involvement and a very limited disease outside the central nervous system (CNS), after a long-lasting remission obtained with conventional treatment with melphalan and prednisone in the first patient and autologous stem cell transplantation in the second one. A cerebral tumor appeared in the other 2 cases who had a disease refractory to standard-dose first-line treatment. At the time of the diagnosis of CNS involvement, PC marrow infiltration of these patients varied between 0 and 40% and no PC were detected in the peripheral blood smear. Clinical symptoms included: mental status changes, leg weakness, cranial nerve palsy, disturbance of gait and speech. Cytological examination of the cerebrospinal fluid (CSF) and CNS magnetic resonance (MRI) were essential for diagnosis. Evidence
THALIDOMIDE IN REFRACTORY AND RELAPSED MULTIPLE MYELOMA

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Introduction. Most patients with plasma cell neoplasia present with bone marrow involvement, multiple or solitary plasmacytomas of the bone or both; increased serum M-component is frequent. Rarely, they may have extramedullary plasmacytoma (EMP) or plasma cell leukemia at onset. Case Report. A 77-year-old man presented in 1999 with right hemithorax pain, lower limb weakness and multiple skin nodules on the back; staging investigations (bone marrow aspiration and trephine, total body CT scan) showed 25% bone marrow plasma-cell infiltration, together with multiple rib, pleural and lung tumor localizations; immunoelectrophoresis revealed an IgA-λ paraprotein. Surgical excision of the fifth and sixth right rib, and of the inferior right lung lobe showed the presence of an IgA-λ multiple myeloma (MM). The patient underwent local radiotherapy and chemotherapy (CT) with melphalan and prednisone attaining a partial response (PR). Fourteen months after diagnosis he presented with 2-10 cm cutaneous infiltrates on the upper trunk and arms, disseminated bone lesions and testicular swelling due to plasmacytoma involvement as documented by ultrasounds and fine needle aspiration. The picture was accompanied by a relevant increase (IgA= 3200 mg/dL) of the serum M-component. Following treatment with, cyclophosphamide, RT on both testicles and diphosphonates, the patients obtained a PR. He is currently in PR, without skin and testicular involvement 21 months after diagnosis. Discussion. In the last 60 years only 48 cases of
Patients affected by multiple myeloma (MM) who present a relapse after chemotherapy at high doses have little therapeutic options. Since the increased medullary vascularity determines a worsening of the prognosis of MM, the use of thalidomide has been recently introduced in an experimental way and, given its antiangiogenetic property, it seems to represent a valid thera-
pic. Ten patients with stage III/III MM were included and, at this time, 7 patients have been remobi-
lized after one course of high-dose melphalan (HDM-I) (200 mg/m²). Median age at transplantation was 59 years (45-70). 9/10 were at first diagnosis and 1 patient received Alexanian for 1 year. The first mobilization scheme consisted in cyclophos-
phamide (4.5 g/m²) and G-CSF (300 mcg/d) started at day +3. Second course of HDCT consisted in Melphalan 200 mg/m² (HDM-II). Results. The medi-
an time between HDM and II mobilization was 4 months (3-
6mo). The median time from II mobilization chemotherapy and first leukapheresis was 14 d (12-17). The median number of CD34+ cells harvested was 2.1×10⁶/kg (0.8-4×10⁶/kg) and medi-
an number of CFU-GM was 41 (21-68)×10⁶/kg. These results were worse compared to median number of CD34+ cells and CFU-GM harvested after first mobilization (13.3×10⁶/kg and 182×10⁶/kg, respectively). However, hematologic toxicity was mild after HDM-II: mean time with neutrophil <500/mcl was 10 days (5-7), and mean time with platelet <20,000/mcl 4.5 days (2-7). Transfusional support was low with a mean number of platelet units transfused of 19 (8-32) and mean number of red cell package 1.6. After HDM-II, all patients received G-CSF (300 mcg/d, sc) starting at day +5. Extra-hematological toxicity (graded by NCI scale) was mainly mucosal: G4 in two patients, G2 and G3 in the others. No patients developed severe infections: one patient had bacteremia (N meningitidis and Staph coag neg) and one patient had a septic arthritis. After the first HDCT, 5/10 pts achieved CR (50%), 4/10 PR (40%) and 1/10 SD (10%). Two patients in PR, obtained CR after second HDCT. Conclusions. Second mobilization after a first course of HDM-I is feasible. Hematopoietic toxicity of HDM-II supported by second leukapheresis was absolutely mild even if CD34+ and CFU-GM were lower than after 1st mobilization. We are analyzing by molecular tools the in vivo purging effect of first HDCT in second leuka-
apheretic product.
Introduction. Osseous plasmacytomas are likely to evolve to overt myelomatosis. Extra-medullary manifestations at progression are, however, rare. Although they can arise in any tissue, breast, pericardium and liver involvement is extremely unusual. Usually they show early response to chemotherapy, followed by soon relapse. Case report. A 57-year old woman was referred in April 1996 for hyposthenia and paresthesias in the lower limbs. Spine MRI showed a pathological tissue at D5 level. Laminectomy was performed, and histologic examination showed plasmacytoma. Serum protein electrophoresis and immunoelectrophoresis demonstrated a monoclonal IgGk protein (1.9 g/dL). Bence-Jones proteinuria was absent. Blood counts showed mild anemia. Calcium, creatinine, polyclonal immunoglobulins, β2-microglobulin and lactate dehydrogenase levels in the serum were normal. No osteolytic lesions was present on skeletal X-ray. Marrow examination demonstrated 7% plasma cells. Osseous Plasmacytoma was diagnosed. Local radiation therapy (30 Gy) and 6 MP cycles induced MRI improvement, but a small-sized serum monoclonal peak (1.4 g/dL) persisted in the absence of radiologic, biochemical or hematologic evidence of myelomatosis. Three months after the end of chemotherapy, pain in the lower limbs appeared. Spine MRI showed plasmacytoma recurrence at S2–S3 level. After local radiation therapy (30 Gy) and 6 alternating CAVD/VCAP cycles, radiologic remission and paraprotein disappearance were noted. Three months after the last CAVD/VCAP administration, the monoclonal peak reappeared. Strict follow-up examinations showed no signs of progression until 8 months later, when an exophytic lesion on the scalp and a palpable mass, 2.5 cm diameter, in the subareolar area of the left breast were noted. Multiple densities and echo-poor solid masses, 2 cm diameter, were present on mammography and breast ultrasound, respectively. CT scan demonstrated a solid mass, 5X9X5 cm diameter, in the antero-inferior left chest wall extending as far as the pericardium, multiple pleural thickenings and a 2 cm hypodense mass in the sixth segmentum lobi hepatitis dex- tri. Echocardiography showed a little pericardial effusion. Fine needle aspiration cytologic finding of the breast and scalp tumors revealed Plasmacytoma. Marrow examination showed 5% plasmablasts; radiographs of the spine, pelvis and skull demonstrated multiple osteolytic lesions. The patient underwent 2 DCEP cycles, with slight mass reduction, and early relapse of the illness (breast mass 7 cm diameter) soon after the end of chemotherapy. We started treatment with Thalidomide (100 mg/day for 10 days, then 200 mg/day), obtaining immediate reduction of breast masses, now almost disappeared. Conclusions. Osseous plasmacytoma can evolve to anaplastic Myeloma with extra-medullary spread and features of dedifferentiation, and extra-osseous localization can be unusual. In the present case, an aggressive chemotherapy (2 DCEP) obtained a transient response, followed by early relapse. Our experience demonstrated an early and dramatic therapeutic effect on extra-medullary masses, with low-dosage Thalidomide (200 mg/die).
PO341
THALIDOMIDE TREATMENT OF RELAPSED MULTIPLE MYELOMA PATIENTS AND CHANGES IN CIRCULATING TNF-α, IL-6, bFGF AND VEGF

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Preclinical studies suggest that thalidomide acts against myeloma in several ways: it inhibits myeloma cell growth, inhibits myeloma cell adhesion to bone marrow stromal cells (BMSCs), blocks IL-6, TNF-α, and IL-1p secretion from BMSCs, blocks the ability of VEGF and bFGF to stimulate neovascularization of bone marrow, and induces IL-2 and interferon gamma secretion from T cells. We present our experience on a small group of patients with stage III myeloma treated with Thalidomide. Six patients had failed stem cell transplantation and four received two or more prior chemotherapy regimens before institution of Thalidomide. The median administered dose of Thalidomide was 600 mg/day. The median time of treatment has been six months. Three patients showed more than 50% reduction in serum M-component concentration, in four patients a decrease > 25% was observed, three patients were refractory. Serum specimens for angiogenic factors were obtained on day 1 immediately before the first dose of Thalidomide. Circulating levels of TNF-α (mean normal population 7.9 pg/mL), VEGF (8.1 pg/mL), and IL-6 (5.1 pg/mL) were determined using ELISA. At baseline the median serum concentrations of bFGF (63.2 pg/mL), normalized VEGF (77.7 pg/mL), TNF-α (37.7) pg/mL, IL-6 (64.6 pg/mL) were significantly increased compared with healthy controls (p=0.000). At week 10 all patients showed a transient increase in the TNF-α level compared with baseline (68 pg/mL). In responding patients, there was a statistically significant reduction in circulating levels of bFGF, VEGF and IL-6.

PO342
FLUDARABINE, CYCLOPHOSPHAMIDE AND DEXAMETHASONE (Flu-CyD) IN NEWLY DIAGNOSED AND PRETREATED LOW-GRADE LYMPHOPROLIFERATIVE DISORDERS

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To evaluate the efficacy and toxicity, a phase II trial was conducted of FluCyD in patients (pts) with untreated and pretreated low-grade lymphoproliferative disorders. Fludarabine 20 mg/m² day 1-5, cyclophosphamide 1000 mg/m² day 1, and dexamethasone 20 mg day 1-5 were given outpatient every four weeks. Full blood count was monitored weekly. Hematopoietic growth factors were not associated unless required by clinical conditions. Previously untreated pts received a total of six courses of FluCyD, whereas those with relapsed disease received four courses. Cases with pretreated disease, as well as those treated frontline and obtaining less than complete remission (CR), were candidates to high-dose consolidation chemotherapy (HDCT) with peripheral-blood stem cell (PBSC) support. From 04/99 to 03/2001, 27 pts have been accrued: 18 males and nine females with a median age of 54 years (range 29-69). Twenty-five pts had a diagnosis of low-grade lymphoma, one chronic lymphocytic leukemia and one lymphoplasmocytoid lymphoma. Fourteen cases received FluCyD frontline, whereas 13 had previously received a median of two chemotherapeutic regimens. Of 22 pts evaluable for therapeutic response, 11 obtained CR, nine partial remission, and two did not respond, with no difference between pretreated and naive pts. Grade IV neutropenia occurred in 13% of 116 evaluable cycles, and grade III-IV thrombocytopenia in 9%. Two pts required red blood cell transfusions, and there was no need for platelet transfusions. Fever of unknown origin of mild to moderate intensity complicated a total of 15 cycles; other extrahematological toxicities were negligible. Three patients experienced grade III infection related to immunosuppression. Ten cycles were delayed or administered at reduced dose due to toxicity. One pt died of untreatable hemolytic anemia two months after completing the treatment program. Up to now, none of three pts candidated to HDCT could collect a sufficient amount of PBSC with standard mobilizing regimens, and in two cases bone marrow harvest was performed. After a median follow-up of 11 months (range 0-22), eight pts have progressed (two induction failures, four relapses after PR, and two histologic transformation), 11 are alive and disease-free, 10 are alive with disease (three treatment ongoing), five have died (one of low-grade lymphoma, two of transformed lymphoma, one of hemolytic anemia, and one of unrelated causes) and one was lost at follow-up. Thus, freedom from progression and overall survival are 59% and 82%, respectively. In conclusion, FluCyD is an effective and well-tolerated regimen for newly diagnosed and pretreated pts with low-grade lymphoproliferative disorders. The incidence of opportunistic infections may be limited by eliminating DEX. The impact of FluCyD on subsequent PBSC mobilization needs further evaluation. The assessment of molecular remission is ongoing.
**P0343**

PROSPECTIVE STUDY OF INDOLENT NON-FOLLICULAR NON-HODGKIN’S Lymphoma: VALIDATION OF THE “GRUPPO ITALIANO PER LO STUDIO DEI LINFOMI” (GISL) PROGNOSTIC CRITERIA FOR WATCH-AND-WAIT POLICY


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In this study, we validated the prognostic criteria proposed by the GISL for indolent non-follicular non-Hodgkin’s lymphoma strictly diagnosed according to histomorphology and immunophenotype to identify patients with favorable non-progressive clinical presentation, thus eligible for a watch-and-wait policy. Fifty-three patients with small lymphocytic, marginal zone and lymphoplasmocytic lymphoma were registered in a prospective therapeutic GISL trial and observed without treatment in case of absence of any of the following features: B symptoms, bulky disease, anemia, thrombocytopenia, diffuse infiltration pattern of bone marrow and short tumor doubling time. After a median follow-up of 41.3 months, the median progression-free survival (PFS) was not reached and 73% of cases did not progress, thus confirming the validity of the GISL definition of indolent disease presentation. When additional prognostic variables were considered, in order to improve the prognostic model, LDH level and number of extranodal sites resulted statistically significant in multivariate analysis. Based on this finding, a prognostic score was devised able to further identify, among cases with indolent disease presentation, a small group of patients more likely to undergo an early progression, thus suitable for immediate treatment. In conclusion, the results of this prospective study confirm that the GISL definition of indolent disease is a reliable tool to design appropriate therapeutic strategies in this histological setting.

**P0344**

AUTOLOGOUS STEM CELL TRANSPLANTATION AS CONSOLIDATION TREATMENT OF AGGRESSIVE NON-HODGKIN’S LYMPHOMA


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Background and Objectives. The role of high dose chemotherapy followed by autologous stem cell transplantation (ASCT) for patients with aggressive non-Hodgkin’s lymphoma (NHIL) in first remission is still a subject of controversy. We report the results of a single center study where ASCT was employed in 93 consecutive patients with aggressive NHL (defined as intermediate or high grade histology and at least one of the following risk factors: Ann Arbor Stage III-IV, bulky disease or B symptoms) and in complete or partial response after induction therapy. Design and Methods. Median age at diagnosis was 39 (15–60) years; male/female ratio - 57/36. They were affected by diffuse large B cell lymphoma - 44 (47.5%); anaplastic large cell lymphoma T or null cell type - 18 (19.5%); T precursor lymphoma - 8 (8.5%); follicular lymphoma - 12 (12.5%); peripheral T cell lymphoma unspecified - 4 (4.5%); marginal zone lymphoma - 5 (5.5%); mantle cell lymphoma - 2 (2.0%); sixty-two out of 93 (66.5%) patients were in stage III-IV, 56 (60.5%) had a bulky disease and 48 (51.5%) had B symptoms. First line therapy consisted of six cycles of chemotherapy according to the F-MACHOP regimen and radiotherapy if a residual mediastinal mass, in a previous bulky disease, was documented. Status at transplant was: complete remission - 58 (62.5%) patients; partial remission - 35 (37.5%) patients; ASCT was performed using BAVC as conditioning regimen (carmustine, cytosine-arabynoside, etoposide, cyclophosphamide). All the patients received G-CSF after ASCT starting from day +4 and for a median of 11 days (10–31). Results. As by March 31, 2001 with a median follow-up from transplant of 64 months (3–127), 92.0% (86/93) of the patients were in complete remission, 3 patients (3.0%) progressed and 1 patient relapsed at a median time from transplant of 36 months (24–60). After ASCT, 29 patients, transplanted in partial response, entered complete remission with a conversion from partial to complete response of about 83.0%. All the 58 patients transplanted in complete remission are still in remission. One death during conditioning regimen was recorded: a patient with anaplastic large cell lymphoma who died of pulmonary vein thrombosis during conditioning, while during transplant follow-up, one secondary acute leukemia was documented. No other major complications were observed, in particular, with a median follow-up from diagnosis of 72 months (10–146) no case of myelodysplastic syndrome has been developed. Conclusions. Our results seems to be in excess of what would be expected in a group of aggressive non-Hodgkin’s lymphoma patients treated with standard chemotherapy only. In particular, what should be underlined is the feasibility of such a program including ASCT as part of first line treatment either in terms of overall and progression-free survival and in terms of long term toxicity.

**P0345**

PRIMARY CEREBRAL LYMPHOMA: REPORT OF 5 CASES

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Introduction. Approximately 3% of HIV–negative patients with diffuse large cell lymphoma (DLCL) involve exclusively the cerebral parenchyma or the meninges or the eye with no tumor outside the central nervous system. These forms were defined primary cerebral lymphoma (PCL). The incidence appears to be increasing. Clinical management of these patients remains controversial. Until 15 years ago the treatment of choice was radiotherapy alone. However, the event-free survival (EFS) and overall survival were very poor. Further studies showed the superiority of a combined approach with chemotherapy plus radiotherapy; more recent trials with high dose chemotherapy seems efficacious. Several authors have reported a relatively high incidence of late neurological sequelae. Patients and Methods. From May 1, 1998 to January 1, 2001, 5 patients with primary DLCL of CNS were treated in our center. The patients with AIDS or who were human immunodeficiency virus (HIV)-positive were not included in this study. Patients with age < 60 y were treated with high dose methotrexate (2 g/m²) and ARA-C (3 g/m² bis die) for 3 days plus intrathecal therapy (ARA-C 40 mg plus prednisolone) followed by whole brain radiotherapy (WBR) 45 Gy; whereas, the patients with age > 60 years were treated with CHOP-like regimen plus WBR. In all patients staging included CT of chest and
PO346
PROSPECTIVE MULTICENTER CLINICAL TRIAL ON THE USE OF INTERFERON-α PLUS PUVA IN PATIENTS WITH EARLY STAGE MYCOSIS FUNGOIDES


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Background and Objectives. The early stages of mycosis fungoides (MF) can be treated but not cured by photochemotherapy (PUVA) alone; some recent studies of the effect of a combination of IFNα and PUVA reported a high degree of response. The aim of our study was to evaluate the activity of a low dose of IFNα combined with PUVA; another objective was to analyze the treatment success in term of freedom from treatment failure (FFT). Design and Methods. Fifty-one patients were included: 30 men and 21 women aged between 29-80 years; 44 patients had stage I (32% in IA, 68% in IB) and 7 stage II disease (86% in IIA-4% in IIB). In the induction phase, the dose of IFNα was gradually raised over 6-8 weeks to the target dose of 18 MU/week; in the maintenance phase, the combination with PUVA allowed IFNα to be reduced to a maximum dose of 6 MU/week. In this way the cumulative administration of IFNα and PUVA was considerably lower than in similar combination protocols, FFT was calculated from treatment initiation to the date of permanent abandoning of treatment for any reason (refusal without adequate reason, lack of efficacy or toxicity of therapy). Results. After the induction phase 23/51 (45%) achieved CR and 26/51 (51%) achieved PR. One to nine months from the beginning of the maintenance phase a CR was recorded in 40/51 patients (78.4%) and a PR in 10/51 patients (19.6%) producing an overall response rate of 98%. The dose schedule of this study was generally well tolerated with only few protocol violation. Follow-up data were available for all patients; the median follow-up time was 32 months. Our results showed that after 12 months of treatment 94% of patients were still event-free; this percent-age decreasing to 71% after 24 months and to 25% after 48 months. With our treatment only one patient with extensive infiltrated plaques progressed to a more advanced stage (IIIB) and none died of disease. All patients who had a relapse cleared (CR or PR) with reinitiation of IFNα therapy (+ PUVA). In conclusion despite the high rate of response observed in PUVA+IFNα treated patients a remarkable number of responding patients relapsed within 48 months. Probably all patients need to continue maintenance therapy indefinitely. It is important to realize that the degree of epidermotropism of T cells decreases during the disease process, thus patients may be less sensitive to PUVA therapy; based on such findings we prefer a maintenance therapy with IFNα. We believe that even low-dose IFNα may be enough to maintain an important antitumor immunity.

PO347
MINIMAL TOTAL BODY IRRADIATION BASED CONDITIONING FOLLOWED BY HLA-IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH LYMHPHOPROLIFERATIVE DISORDERS

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The main obstacles in expanding the use of conventional allogeneic stem cell transplantation (ASCT) in lymphoproliferative disorders are that morbidity and mortality remain quite high and that many patients are deemed ineligible because of their age and/or clinical condition. The use of less toxic, non-myeloablative preparative regimens leads to a safer transplant procedure without affecting the development of graft-versus-tumor effect, and the good efficacy of the combination of fludarabine and cyclophosphamide (CTX) in advanced LD can lead to the degree of immunosuppression required for stem cell engraftment. Five patients with B-CLL, 2 with follicular lymphoma (FLC) and 3 with Sézary syndrome (SS) were transplanted after having previously received more than two lines of conventional chemotherapy. At the time of transplant, 3 of the CLL patients were in stage C and 2 in stage IVB; the two FCL and the 3 SS were stage IVA. Two cycles of fludarabine 30mg/m²/day for 3 days and CTX 300 mg/m²/day for 3 days (FC) were repeated every 28 days; fourteen days after the second cycle, TBI 200 cGy was delivered as a single dose, followed by an HLA-identical sibling donor PBSC infusion. GVHD prophylaxis consisted of cyclosporin-5 mg/kg b.i.d. p.o. from day -1 to +90 and mycophenolate mofetil 15 mg/kg b.i.d. p.o. from day 0 to +27. Two patients experiencing rejection underwent a second non-myeloablative ASCT, which was followed by full donor engraftment on day +28. The major post-transplant complications were one case of CMV pneumonia, one case of alloimmune hemolytic anemia associated with severe thrombocytopenia, and one case of EBV-related meningoen-cephalitis. Five patients required red blood cell and platelet transfusions. No grade II acute GVHD was observed and all patients are alive after a median follow-up of 12 months. Of the 9 evaluable patients, 6 achieved CR (2 FCL, 2 CLL, 2 SS), and 3 a PR (3 CLL). The conditioning used in this series of patients can be considered minimal even among non-myeloablative regimens, and so the achievement of a response mainly relied on the
expected development of an effective GVL. Despite the low-intensity of the regimen, donor engraftment was reached in all of the patients, although graft rejection subsequently occurred in two. A particular feature in this series was the absence of acute GVHD and the fact that disease control was recorded in all cases at least until donor engraftment was detectable. Since post-ASCT antineoplastic efficacy continued far beyond any possible chronological relationship to conditioning regimen, it can be inferred that the procedure induced a GVL effect other than GVHD. However, other severe events complicated post-transplant course, some of which were rather unusual and possibly related to the type of conditioning, including alloimmune hemolytic anemia. Non-myeloablative ASCT should therefore not be considered a clinically trivial procedure.

PO348
MITOXANTRONE, PREDNISONE, PENTOSTATIN, AND BLEOMYCIN
(MiPPeB) FOR PATIENTS WITH RELAPSED INDOLENT
NON-HODGKIN’S LYMPHOMA

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Indolent NHL includes different entities characterized by similar clinical course, with relatively good prognosis in terms of overall survival. Although patients with indolent NHL usually respond to initial therapy, they exhibit a tendency to relapse, with subsequent responses of progressively shorter duration. In patients with recurrent or relapsed indolent NHL response rates of 40-60% have been reported with different approaches, including doxorubicin containing regimens, high dose therapy, anti-CD-20 monoclonal antibodies and purine nucleoside analogs. Among these, pentostatin has been relatively less employed for the treatment of indolent NHL although its efficacy has been demonstrated in different lymphoproliferative disorders such as HCL, Mycosis Fungoides, Sézary syndrome and prolymphocytic leukemia. Taking into account the promising results achieved with purine analogs in combination regimens in patients with indolent NHL, we designed a phase II study aimed to assess the efficacy of pentostatin in combination with mitoxantrone, prednisoni and bleomycin. Patients were eligible for the study if they presented with indolent NHL categories A-D of the WF, or lymphocytic or follicular lymphoma of the R.E.A.L.), relapsed after previous therapy, in stage II-IV and with active disease. MiPPeB regimen included 4 drugs (mitoxantrone 10 mg/m2 on day 1, pentostatin 5 mg/m2 on days 1 and 8, predniione 100 mg on days 1 and 8 and bleomycin 8 mg/m2 on day 8) given every 3 weeks for a total of 6 courses. Between November 1996 and July 2000, 30 patients (18 males and 12 females) were enrolled. Nine, 10 and 11 patients had already received one, 2 or 3 or more lines of therapy respectively. A median of 5 cycles (range 2-6) was administered. In the 29 patients evaluable for response, 9 CRs and 8 PRs were observed, with an overall (CR+PR) response rate of 59%. After a median follow-up of 16 months the 3 year overall and failure free survival were 60% and 20% respectively. The actuarial 3-year relapse free survival for 9 patients in CR was 51%. Treatment was well tolerated in the majority of cases. Toxicity was mainly hematologic with grade 3-4 neutropenia in 37% and grade 3 thrombocytopenia in 7%. In conclusion MiPPeB has been proven to be an active regimen for patients with relapsed indolent NHL and resulted in lasting remissions. Moreover, the regi-
ment was well tolerated, with minimal toxicity.

PO349
MACOP-B REGIMEN FOLLOWED BY INVOLVED FIELD RADIATION THERAPY
IN EARLY STAGE AGGRESSIVE NON-HODGKIN’S LYMPHOMA PATIENTS:
14 YEAR UPDATE RESULTS

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A single center, retrospective study was conducted to evaluate therapeutic results of the MACOP-B third generation chemotherapy regimen followed by involved-field radiation therapy in stage I-II aggressive non-Hodgkin’s lymphoma (NHL) patients. From 1986 to 1995, 118 consecutive patients with the diagnosis of aggressive NHL, stage I-II or III-IE, with or without bulky disease were treated with the MACOP-B regimen followed, when appropriated, by 30-36 Gy involved-field radiation therapy. The complete response (CR) rate was 95% after the combined modality treatment (97% for stage I-II and 93% for stage III-IE). Patients with bulky disease had a CR rate of 92%. Treatment was well tolerated and no deaths occurred from acute toxicity. After a median follow-up of 68 months, 24 (21%) patients relapsed. The 14-year projected relapse-free and overall survival rates were 78% and 69% respectively. MACOP-B regimen with or without involved-field radiation therapy provides a safe and effective combined modality treatment for early-stage aggressive NHL with the possibility of definitively curing two thirds of the patients.

PO350
ANALYSIS OF LYMPH NODE INVOLVEMENT IN NON-HODGKIN’S
LYMPHOMAS BY MULTIPARAMETRIC FLOW CYTOMETRY

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Background: Recently multiparametric flow cytometric analysis (MF) is being used in addition to histologic analysis for a more precise evaluation of lymph node involvement in non-Hodgkin’s lymphomas (NHL). This technique allows a more quickly diagnosis (result in the same day of the biopsy) and permits to start an early treatment. Patients and methods. Between April 2000 and March 2001, 21 patients (9 male and 12 female with a median age of 53 years, range 21-84 years) with a suspected diagnosis of lymphoma were submitted to a lymph nodal biopsy. The nodal specimens after collection were histologically analysed on hema-
Conventional treatment of elderly patients with high-grade non-Hodgkin's lymphoma (HG-NHL) is disappointing due to the high percentage of treatment-related toxic deaths and to a sub-optimal dose intensity of treatment. We report this intensive treatment for advanced aggressive NHL for the elderly patients with: vincristine 1.4 mg/m² and epirubicin 120 mg/m² on day 0 and +45, cyclophosphamide 4 g/m² on day +15 and +60, and etoposide 1.5 g/m² on day +30 and +75; the drugs were administered sequentially for a total of six doses. Filgrastim was delivered after each course to PMN > 500/µL. Involved field radiotherapy was given for bulky lesions. From February 1995 to May 2000 20 patients with HG-NHL were consecutively enrolled in a phase II trial; 18 out of 20 were at diagnosis, 2 patients were in first relapse. The median age was 68 years (range, 62 to 73 years), 11 patients were male and 9 females. Stage was II in 5 patients, III in 4 patients, IV in 11 patients. Two patients had extranodal disease associated with nodal presentation. Age adjusted IPI (aIPI) was as follows: 0 in 1 patient, 1 in 8 patients, 2 in 9 patients, 3 in 2 patients. Histology was diffuse large B cell NHL in 19 cases, and peripheral T cell NHL in 1 patient. Thirteen out of 20 patients achieved CR (65%), 5 achieved PR (25%), 2 patients (10%) had progressive disease. Seven out of 13 CR patients have relapsed after a median of 8 months (range, 3-29 months), and 3 of them are alive in second or further CR. Five out of 20 patients underwent peripheral blood stem cell transplant (PBSCT): 2 in first CR, 2 in PR and 1 in second CR. Conditioning regimen included mitoxantrone and melphalan, with doses ranging respectively between 45 and 60 mg/m² and between 90 and 140 mg/m². After PBSCT all the patients are alive, 4 out of 5 achieved CR and 1 very good PR. After a median follow-up of 23 months the DFS for the entire group of pts was 50% and the OS 55%. The hematologic toxicity, consisting of grade 3-4 neutropenia and thrombocytopenia, was recorded in all patients. Extra-hematologic toxicity included alopecia in all, grade 2 arrhythmia in 3 patients, grade 2 pneumonia in 3 patients, grade 3 acute cholecystitis in 1 case, and grade 4 polyneuropathy in 1 case. No toxic death occurred. In summary, the very low toxicity of this short intensive regimen warrants its feasibility in the elderly patients; the preliminary results in terms of DFS and OS are appealing, considering the very poor prognosis of these patients (aIPI 2-3 in 12 out of 20 pts.) and seem to indicate that this program should be tested in a larger multicenter trial.

PO351
HIGH-DOSE SEQUENTIAL CHEMOTHERAPY FOR HIGH-GRADE NON-HODGKIN'S LYMPHOMA IN ELDERLY PATIENTS

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Conventional treatment of elderly patients with high-grade non-Hodgkin's lymphoma (HG-NHL) is disappointing due to the high percentage of treatment-related toxic deaths and to a sub-optimal dose intensity of treatment. We report this intensive treatment for advanced aggressive NHL for the elderly patients with: vincristine 1.4 mg/m² and epirubicin 120 mg/m² on day 0 and +45, cyclophosphamide 4 g/m² on day +15 and +60, and etoposide 1.5 g/m² on day +30 and +75; the drugs were administered sequentially for a total of six doses. Filgrastim was delivered after each course to PMN > 500/µL. Involved field radiotherapy was given for bulky lesions. From February 1995 to May 2000 20 patients with HG-NHL were consecutively enrolled in a phase II trial; 18 out of 20 were at diagnosis, 2 patients were in first relapse. The median age was 68 years (range, 62 to 73 years), 11 patients were male and 9 females. Stage was II in 5 patients, III in 4 patients, IV in 11 patients. Two patients had extranodal disease associated with nodal presentation. Age adjusted IPI (aIPI) was as follows: 0 in 1 patient, 1 in 8 patients, 2 in 9 patients, 3 in 2 patients. Histology was diffuse large B cell NHL in 19 cases, and peripheral T cell NHL in 1 patient. Thirteen out of 20 patients achieved CR (65%), 5 achieved PR (25%), 2 patients (10%) had progressive disease. Seven out of 13 CR patients have relapsed after a median of 8 months (range, 3-29 months), and 3 of them are alive in second or further CR. Five out of 20 patients underwent peripheral blood stem cell transplant (PBSCT): 2 in first CR, 2 in PR and 1 in second CR. Conditioning regimen included mitoxantrone and melphalan, with doses ranging respectively between 45 and 60 mg/m² and between 90 and 140 mg/m². After PBSCT all the patients are alive, 4 out of 5 achieved CR and 1 very good PR. After a median follow-up of 23 months the DFS for the entire group of pts was 50% and the OS 55%. The hematologic toxicity, consisting of grade 3-4 neutropenia and thrombocytopenia, was recorded in all patients. Extra-hematologic toxicity included alopecia in all, grade 2 arrhythmia in 3 patients, grade 2 pneumonia in 3 patients, grade 3 acute cholecystitis in 1 case, and grade 4 polyneuropathy in 1 case. No toxic death occurred. In summary, the very low toxicity of this short intensive regimen warrants its feasibility in the elderly patients; the preliminary results in terms of DFS and OS are appealing, considering the very poor prognosis of these patients (aIPI 2-3 in 12 out of 20 pts.) and seem to indicate that this program should be tested in a larger multicenter trial.
marrow and he also relapsed. A further relapse was recorded in a patient with FCCL. The follow up of patients who are still in remission is between 14 and 26 months. The five patients entered in the second program are all in clinical and immunological remission despite not having completed the therapy; the remission was obtained following the first mobilization and first rituximab in 4 patients and following the second rituximab in one. The follow up of patients who completed the program is between 4 and 9 months. Our conclusion is that a program with intensive and high dose therapy including rituximab in CD20 lymphomas with marrow involvement gives the possibility of reaching immunologic remission in most patients. Longer follow up will answer if rituximab could increase the possibility of cure of such patients.

**PO355**

FIRST LINE THERAPY WITH VEMP REGIMEN IN ELDERLY HIGH GRADE MALIGNANCY NON HODGKIN'S LYMPHOMAS

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In the management of elderly patients affected by high grade malignancy non-Hodgkin’s lymphomas (HGM NHL), the goal should be to apply regimens with less toxicity, but same efficacy to standard chemotherapy for the age and the presence of concomitant disease, one day delivery makes it easy to apply in outpatient's or during short hospitalization.

**PO354**

2-CHLORO-DEOXY-ADENOSINE INDUCED COMPLETE REMISSION IN PRIMARY UNIFOCAL LANGHERANS’ CELL HISTIOCYTOSIS OF THE CENTRAL NERVOUS SYSTEM: REPORT OF A CASE

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The term Langherans’cell histiocytosis (LCH) designates a whole of several lesions characterized by proliferation of particular cells defined as Langherans’ cell. The lesions are due to the clonal proliferation of dendritic antigen-presenting cells (DC) that are mainly localized in the skin, but can also be found in other tissues. The primary CNS LCH, especially of the brain stem, is a rare condition, up to date very few cases have been reported. 2-chlorodeoxyadenosine (2-CDA, Cladribine), a purine analogue, active in indolent lymphoproliferative disorders and myeloid leukemia is in vitro powerful toxic agent on monocytes. Because tissue histiocytes and circulating monocytes have common progenitor cell, Cladribine is a rational therapeutic option. We describe here the case of a 38-year-old woman presented at our institution with a history of nausea and vomiting episodes. The brain computed tomography (CT) scan did not reveal any lesion, while magnetic resonance (MR) showed a solitary mass (1.5 cm -diameter expansible) originating within the lateral wall of the fourth ventricle. The patient was observed until September 1997. At that time the patient began to develop a progressive marked right-sided ataxia and tremor, a second MR was performed in September 1997 revealing a voluminous mass (3 cm-diameter) increased respect to the last MR in January 1998 a stereotactic biopsy was performed and a LCH diagnosis was established. In February 1998 the patient underwent elsewhere a program of palliative cranial radiotherapy (total dose 30 Gy). In July 1999 the patient was again referred to our institution: she was lethargic, marked ataxia, tremor, dizziness were present. MR showed a voluminous enhancing mass (>3 cm-diameter). The patient started Prednisone treatment (1.5 mg/kg/daily) and an initial improvement of neurological status was observed. From November 1999 until June 2000 patient agreed to be treated with 2-CDA 5mg/m2/day for five days for six courses. After the first course an improvement of clinical status was evidenced increasing until the sixth course. At that time neurological examination was negative but MR imaging after third and sixth course persisted unchanged. A localized dermal herpes Zoster infection, which healed in few days was the only one observed side effect. In December 2000, six months off-therapy, MR imaging showed a near-disappearance of the lesion. Up to date the last MR and neurological examination confirmed a complete remission. Our report is, to our knowledge, the first description of a primary unifocal Langerans’ cell histiocytosis of the CNS treated with Cladribine obtaining a complete response. 2-CDA demonstrated an effective and safe agent; the patient obtained both complete clinical recovery and radiological disappearance of CNS localization. The schedule applied was well tolerated, no hematological toxicity was observed except the reduction of lymphocyte absolute value not translating in an increased incidence of infection.

**PO355**

FEASIBILITY OF AN ORAL CHEMOTHERAPY REGIMEN (CIEP) IN THE TREATMENT OF DIFFUSE LARGE CELL NON HODGKIN'S LYMPHOMA IN ADVANCED STAGE IN ELDERLY PATIENTS. PRELIMINARY REPORT FROM ITALIAN LYMPHOMA INTERGROUP


Italian Lymphoma Intergroup (ILI)

Objectives. To value the feasibility and the response rate of an oral chemotherapy regimen in a population of elderly patients...
affected by diffuse large cell lymphoma in advanced stage. The intent is to reduce the days of hospitalization also as outpatients and to improve the quality of life. Patients and Methods. From April 2000 to April 2001 30 patients over 65 years old were enrolled in the study from 14 different onco-hematological institutions. They were treated with oral regimen CIEP: Cyclophosphamide 200 mg/m² days 2–6; Idarubicin 8 mg/m² days 1,3,5; Etoposide 100 mg days 2,4,6; Prednimase 50 mg days 1–5. The total number of cycles is six. The frequency is every 21 days. At the end, radiotherapy on residual masses is optional. Oral idarubicin is provided from Pharmacia. Results. We have registration data of only 26 out of 30 enrolled patients. Median age is 77 (65–91). Ratio male-female is 10/16. This is the stage division: 5 patients were in stage II with a bulky mass; 13 (50%) were in stage III; 8 (31%) were in stage IV. Therapy was well tolerated. There was only a case of suspected toxic death (a severe arrhythmia occurred in the course of treatment). Nobody stopped therapy for intolerance (a patient 91 years old spontaneously stopped therapy after 2 cycles because she had a complete remission). We registered 2 cases of progression in the course of therapy. Only a few cases (11) can be evaluated for response, because mostly patients are in treatment, now. We have obtained 3 CR and 4 PR. Conclusions. CIEP regimen seems feasible and well tolerated. The major issue is represented by the high number of tablets which the patients must take every day of the cycle, in particular because Cyclophosphamide is available only in low dosage formulation (50 mg). This issue is more relevant in elderly patients because they often take oral therapy for frequent comorbidity.

PO356

PROBLEM-SOLVING WITH SMALL-GROUP TUTORIALS: A USEFUL TOOL FOR CONTINUING MEDICAL EDUCATION


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Rationale. According to WHO guidelines, continuing medical education should be closely into line with health needs and learned-centered. Moreover, training programmes should give the opportunity to learn how to solve problems in a multiprofessional way. These considerations gave rise to the idea to carry out an Educational Course for Hematologists and Pathologists, in close collaboration with both the Scientific Societies, supported by the CEFPAS’s center of Caltanissetta (Sicily). The main goals of the Course, held in Caltanissetta were the following: to provide advanced knowledges in the field of non-Hodgkin’s lymphomas; to improve the attitude of correlating clinical, morphological, phenotypic and biological data as a means for developing and using formal decision trees; to encourage the different specialists involved, as a team, in the management of a patient affected by malignant lymphoma to share decision responsibilities, so enhancing health care quality. Course Planning and Making. The course structure comprised six tutorial, centered on small-group tutor assisted learning with the method of problem-solving, using simulated cases. A plenary session followed for reporting back group work, for appraisal, demonstration of understanding of the problem and satisfactory response to the learning issues and consultation with the experts (a pathologist and a hematologist) about the critical items. Academic lectures by the experts concluded the session. The fifty attendants, admitted according to pre-defined criteria, were provided with educational handouts, for independent study. The teaching-team (equally distributed between Hematologists and Pathologists) included tutors and experts, with recognized professional skills in the different issues examined. Course preparation consisted in the following steps: survey of the educational needs; definition of the learning issues (proper use of the proposed WHO classification of lymphoid neoplasms; standardization of flow-chart for differential diagnosis, according to Evidence-Based Medicine; staging and therapeutic approach planning according to quality standard and cost/benefit ratio; evaluation of response to therapy); tutors’ training; planning of performance indicators to assess achievement of organization given objectives (interest for issues, usefulness of gained learning ability, achievement of learning goals, efficacy of educational methods used, efficacy of the course, adequacy of course length, active role of attendants, usefulness of distributed educational material, organisation and management adequacy); preparation of self-assessment instruments to check the learners’ gain proficiency. Results and conclusions. The attendants satisfaction index, in terms of perceived quality and scored by the evaluation questionnaire at the end of the course, was altogether of the 84% (67–96%). The learning evaluation, scored by the confrontation between the pre- and post-test results, shows a significant increase in the percentage of correct answers. The results of this educational experience strongly suggest that the Problem-solving with small-group tutorial represents an efficient and effective educational method to optimize continuing medical education processes.

PO357

FLOW CYTOMETRY IN THE DIAGNOSIS OF NON-HODGKIN’S B-CELL LYMPHOMAS: A PROPOSED SCORING SYSTEM


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We evaluated the sIgk/sIgλ ratio in 357 tissue suspensions suspected of being involved by lymphoma (279 lymph nodes, 21 spleen, 8 tonsils, 49 other) by means of flow cytometry (CFM); 25 cases were unevaluable because of CD19- lymphocyte percentage ≤ 5% (14 cases: 3.9%) or the absence of sig expression (11 cases, 3.1%). Of the 332 evaluable cases, 226 had a histological diagnosis of B-cell lymphoma (B-NHL). Light chain restriction (sIgκ/sIgλ ratio ≤ 0.5 or > 4.0) was demonstrable in respective-ly 73 (32.3%) and 141 cases (62.4%), whereas the ratio was conserved in 12 cases (5.3%). In the remaining 106 cases with a histological diagnosis other than B-NHL, light chain restriction was demonstrable in five (4.7%), although three of these were histologically diagnosed as having B-NHL on the basis of a sub-
A prospective randomized GISL trial (LL01) for the treatment of non-follicular small B-cell lymphomas

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In July 1993, GISL proposed a protocol for the study and treatment of low-grade non-Hodgkin lymphomas of extra-follicular origin and related leukemias. This prospective randomized study involved pts with a histological or immunocytomorphological diagnosis (on blood and/or bone marrow samples) of lymphocytic lymphoma, lymphoplasmocytoid lymphoma/immunocytoma, B-monocytoid/marginal zone lymphoma, and splenic lymphoma with villous lymphocytes. Patients with classic CLL were ineligible. Treatment was given to pts in stage III, IV and II with more than three involved sites, and those with active leukemia, defined as the presence of at least one of the following: B symptoms, bulk, anemia, thrombocytopenia, lymphocyte doubling time <12 months or a volumetric increase in at least three nodal sites.

Group A received HDChl-P, chlorambucil 15 mg/m2 /day and prednisone 100 mg/day p.o. for 5 days every 28 days; group B received HDChl-PE, HDChl-P + epirubicin 60 mg/m2 i.v. on day 1. In the case of CR or PR after three cycles, the treatment was continued for a further five cycles; in the case of SD or PD, the pts on HDChl-PE were switched to CEOP and those on HDChl-PE to FAMP. At the end of eight cycles, the pts were randomized to IFN maintenance treatment vs observation. The aims of the study were to evaluate: a) the validity of the proposed criteria for defining indolent disease; b) the effect of epirubicin on therapeutic response; c) the effect of IFN on response duration; d) the efficacy of FAMP as second-line treatment; e) the behaviour of pts with a histological vs immunocytomorphological diagnosis. As of 1 April 2001, 217 pts had been registered in the study: 164 with active and 53 with indolent disease. Among the patients with active disease, only 133 were considered eligible for data evaluation (mean age 62.2 yrs, M:F ratio 66/67), 93 with a histological and 40 with a cytological diagnosis. More than 90% were in stage IV: B symptoms were present in 21 pts and increased serum LDH levels in 42. The median follow-up is now 32.2 months (2-93). Among 114 evaluable pts, induction therapy led to 33 CR (28.9%), 50 PR (43.9%) and 31 SD/PD (27.2%). There do not seem to be any differences relating to the type of therapy (CR+PR in 42 group A pts vs 40 group B pts) or the type of diagnosis. No particularly severe WHO toxicity was observed. In responsive pts, 35 relapses/progressions were observed with a median Time To Progression of 42 mos. independently of the treatment arm.

PO359
PRIMARY SPLENIC LYMPHOMA IS VERY FREQUENTLY A HEPATITIS C VIRUS-RELATED LYMHPHOPROLIFERATIVE DISEASE

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During the past ten years we have observed 11 patients with Primary Splenic Lymphoma (PSL), defined as histologically proven non-Hodgkin’s lymphoma (NHL) in the removed spleen in absence of other NHL localisation (no superficial nor deep-seated lymph nodes by US or CT scan, absence of bone marrow involvement by flow cytometry and bone biopsy). They were 7 males and 4 females, age ranging 46-70 (median 57). At splenectomy, spleen size ranged 0.16 to 1.8 kg (median 1 kg); macroscopically, spleen pattern was nodular in 10 cases and diffuse in one. Histological grading (WF and REAL) was considered low in 2 cases, intermediate in 2, high in 7. When tested for HCV antibodies, 9 out of the 11 patients were found to be positive, while all were negative for HBV antigens and antibodies. Of the 9 HCV+ patients, 5 had chronic active liver disease, 2 had cirrhosis, the other two had no sign of liver disease. After splenectomy, 10 patients received chemotherapy, which was well tolerated; one patient was not treated. All patients are surviving with a follow up ranging 0.1 to 10.5 years (median 3.7 year). Spleen as the primary and unique site of NHL is rarely reported. We would like to stress two aspects of PSL which emerged from our series: the exceedingly high prevalence of HCV infection and the favorable prognosis following adequate treatment. As for HCV, a prevalence of infection in PSL of about 80% (9 out of 11 cases) largely exceeds that observed for nodal NHL even in the geographic areas from where the highest prevalence has been reported (Italy 10-20%). Such a high prevalence suggests that HCV is involved in splenic lymphomagenesis, either directly or indirectly. This suggestion is supported by the finding that even other lymphomas with predominant spleen involvement (SLVL, WM) have shown the highest HCV infection prevalence. It remains to be clarified why splenic lymphocytes should be proner
GLANDS: REPORT OF 20 CASES

MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA OF THE SALIVARY GLANDS: REPORT OF 20 CASES

PO360


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Salivary glands represent one of the main sites of involvement of non-gastrointestinal MALT Lymphomas. Primary salivary gland MALT lymphoma is a rare disease, often associated with autoimmune diseases (e.g. Sjögren syndrome) and HCV infection, and data regarding their clinical behaviour are scanty. Twenty cases of primary salivary gland MALT Lymphomas (2 males and 18 females, aged 21 to 75 years - median 60 years) had been diagnosed in our Institutions between July 1989 and February 1999. The histological diagnosis was made according to the REAL/WHO Classification. In 19/20 pts. parotid gland was involved (in 6 cases the involvement was bilateral), in 1 case a minor salivary gland. The infiltration of a cervical lymph-node and bone marrow was present in 1 and 2 pts respectively. Staging according to Lugano System was: stage I = 17 pts, stage II = 1, stage IV = 2. Previous history of Sjögren syndrome, and Helicobacter Pylori-positive gastritis were present in 4 and 5 pts respectively. HCV Ab were positive in 5 of the 16 evaluated pts. Treatment consisted in: local surgery in 10 pts, + radiotherapy in 2, + chemotherapy in 2), chemotherapy in 7 (+ radiotherapy in 2, + ifN in 1), RT alone in 2, no therapy in 1. CR was achieved in 11 cases, PR in 3, stable disease in 5. One pt. was not evaluable. The median follow-up is 58 months (range 6-110); 17/20 pts (85%) are alive. Histological transformation in high grade lymphoma occurred in 2 pts who died of disease progression. One pt. died of unrelated causes. In conclusion, our data confirm that salivary gland MALT lymphoma is a rare disease occurring preferentially in women and often associated with Sjögren syndrome and HCV infection. Although the transformation in high grade lymphoma is possible, the behaviour is mostly indolent. Local treatment (surgery, radiotherapy) seems to be adequate in most cases.

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INCREASED INCIDENCE OF HEPATIC INVOLVEMENT IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA AND HEPATITIS C VIRUS INFECTION

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Several studies suggest a possible role of hepatitis C virus (HCV) in the pathogenesis of non-Hodgkin’s lymphoma (NHL). This association seems to be present only in some geographical areas, for B-cell NHL, and with preferential extranodal localizations. Few cases of primary hepatic NHL with concomitant HCV infection have already been reported. We describe here clinical and pathological features of HCV-positive NHL patients with liver involvement. We retrospectively analyzed NHL patients tested for HCV antibodies, without any obvious risk factor for HCV infection, seen in our Institution between 1991 and 2000. We identified 63/355 patients (18%) who were positive for HCV-antibodies (Ab) tested by ELISA and RIBA. Eight of the 63 positive patients (13%) presented with lymphoma involving the liver. Of interest, in patients who were negative for HCV-Ab hepatic involvement was significantly less frequent (7/292) when compared to HCV-positive patients (p<0.01). Of these 8 patients, 4 were males, and median age was 70 years (range 52-81). All these patients had positive RT-PCR for HCV-RNA sequences, documenting an active viral infection. Cryoglobulines were positive in half of the patients. By ultrasounds or liver biopsy, 4 patients had cirrhosis, and 3 had chronic hepatitis at the time of diagnosis of lymphoma. Diagnosis of NHL was made on liver biopsy in 6, and on bone marrow and lymphnode biopsy in the remaining 2. Liver specimens were classified as diffuse large B-cell lymphoma (B-DLCL) in 4, and as follicular center cell lymphoma (FCCL) in 2. The 2 other patients, both with B-DLCL, had clinical and radiological evidence of liver involvement by the tumor. Two patients had primary hepatic B-DLCL and were classified as Ann Arbor stage IE. All other patients had stage IV because of bone marrow involvement (5), diffuse lymphadenopathy (3), and spleen involvement (4). Hepatic involvement consisted of a single lesion in 5 cases, and multiple lesions in 3. At the time of diagnosis LDH was elevated in all patients, while AST and ALT were abnormal only in 2 cases. Only one patient did not receive any treatment due to advanced age and low performance status, while the remaining 7 were treated with CHOP or CHOP-like regimens without any complication due to HCV infection. None of the treated patients had to stop the treatment because of liver function impairment. We conclude that in our NHL series hepatic involvement was significantly more represented in HCV infected patients, and concomitant involvement of spleen and bone marrow was frequent. This observation needs further investigations, in relation to the possible role of this hepatotropic and lymphotropic virus in lymphomagenesis.
PO362
ANTIBIOTIC THERAPY OF LOW-GRADE GASTRIC MUCOSA-ASSOCIATED LYMPHOID TISSUE LYMPHOMA: STUDY OF 51 PATIENTS

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Although cure of Helicobacter pylori (HP) infection has given good remission rate in low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma patients, it remains unclear which patients stand to benefit from front-line treatment with antibiotics. 51 low-grade gastric MALT lymphoma patients with stage IE were followed after treatment of HP infection with up two regimens of antibiotics in a sequential study. All patients were monitored by endoscopic ultrasonography during the follow-up. Of the 51 patients, 45 (88%) achieved eradication of HP. Complete MALT lymphoma regression was observed in 31 (61%) patients with a median time required to achieve histologic regression of 6 months (range 1-14 months). Four of these 31 (13%) patients were still positive for HP at the end of antibiotic treatment. Only 3 out 31 (10%) had a local relapse within the first 20 months: in all these cases tumor progression was defined by endoscopic evaluation. Tumors in the distal stomach were associated with more favorable response than those in the proximal stomach or with diffuse infiltration. With a projection at 42 months, the relapse-free survival curve was 88%. A specific subset of low-grade gastric MALT lymphoma patients, including stage IE with proximal or distal infiltration, seem to be the best candidates for cure with the antibiotics alone. In some patients, evidence of HP at diagnosis or relapse is probably not necessary for these lymphomas to respond to antibiotics. For restaging and follow-up, endoscopic ultrasonography can play a pivotal role for evaluation of any lymphomatous disease.

PO363
FOCAL LIVER LESIONS IN NON-HODGKIN’S LYMPHOMA: PREVALENCE, CLINICAL SIGNIFICANCE AND ROLE OF HEPATITIS C VIRUS INFECTION

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The imaging techniques, like ultrasonography (US) or computed tomography (CT) allow complete liver scanning and the accurate detection of the focal lesions in the liver parenchyma. The occurrence of such lesions in a patient with non Hodgkin’s lymphoma (NHL), both at onset of the disease or during the follow-up, is of great significance, because it affects staging, prognosis and therapeutic choices. Moreover, the occurrence of focal liver lesions in patients with lymphoma is generally regarded as a liver involvement of the disease. Nonetheless, data on the prevalence and clinical significance of focal liver lesions occurring in these patients are fragmentary and very poor. Therefore, we evaluated retrospectively the prevalence, nature and clinical significance of focal liver lesions diagnosed with imaging techniques (ultrasonography and computed tomography) in a series of 414 consecutive patients with NHL. The pathologic nature of the lesions was established by ultrasound (US) guided biopsy, evaluation of the response to chemotherapy for the underlying disease and clinical and US follow up. Subtype of NHL (aggressive or indolent), Hepatitis C virus (HCV) status and occurrence of second malignant tumours were also considered. Among our 414 cases, were detected 129 focal liver lesions (76 at onset and 53 during the follow up). Hepatic involvement by NHL was diagnosed in 69 cases (53.5%: 30 at onset and 39 during the follow up) and it was more frequent in patients with aggressive NHL. Neoplastic, non NHL lesions were detected in 10 cases, including 7 cases of Hepatocellular Carcinoma (HCC) and 3 of metastasis. The remainder 50 focal lesions were benign. All the cases of HCC occurred in HCV positive patients. Excluding HCC, no differences were found in the prevalence and pathologic nature of focal liver lesions and occurrence of second malignant tumours between HCV negative and HCV positive NHL patients. We conclude that the focal liver lesions detected in NHL patients are frequently unrelated to the underlying disease. Consequently, all lesions detected in these patients should undergo a complete diagnostic work up. Finally the not rare occurrence of HCC in HCV positive patients, must be kept in mind.

PO364
HD-MACHOP (INVOLVED FIELD RADIOTHERAPY) AND AUTOLOGOUS STEM CELL TRANSPLANTATION AS FRONT LINE THERAPY FOR PATIENTS WITH HIGH-INTERMEDIATE AND HIGH RISK NON-HODGKIN’S LYMPHOMA

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Introduction. Patients with high-intermediate (HI) or high (H) risk non-Hodgkin’s lymphoma (NHL) according to the International Prognostic Index (IPI) have a poor outcome, with a 5-years projected survival of 40% and 30 %, respectively. Herein we report the results of an ongoing prospective single centre clinical trial for patients with HI and H IPI risk NHL, based on the administration of an intensified third generation induction regimen (HD-MACHOP), RT on initial bulky or localised post induction residual disease, peripheral blood stem cells collection (PBSC) and autologous stem cell transplantation (ASCT). Patients and methods. Inclusion criteria were the following: age less than 60 years old, HI or H IPI risk, high risk histology (with the exclusion of precursor B or T-cell and mantle cell NHL), no previous treatment, HIV negativity, absence of other concomitant important diseases. The induction therapy with HD-MACHOP consists in the administration of HD-MACHOP/A (prednisone 60 mg/m² day 1 to 14, vincristine 2 mg/m² day 1, adryamicin 60 mg/m² day 2, cytosine- arabinoside 1000 mg/m² day 2, methotrexate 1500 mg/m² day 3) alternated to HD-MACHOP/B (prednisone 60 mg/m² day 1 to 14, vincristine 2 mg/m² day 1, ifosfamide 800 mg/m² day 2 and 3, adryamicine 60 mg/m² day 2, cytosine- arabinoside 2000 mg/m² day 2 and 3, methotrexate 500 mg/m² day 3) to be repeated every 21 days for 6 courses. Patients in complete (CR) or partial remission (PR) undergo PBSC collection with G-CSF after the fourth or fifth HD-MACHOP or within three months after the last HD-MACHOP course. ASCT is performed using BAVC as conditioning regimen (carmustine, cytosine-arabonoside, etoposide, cyclophosphamide). Results. At
present 18 patients (14 HI and 4 H risk), median age of 55 years (range 20-55 years), have been registered into the study. Fifteen patients completed the induction therapy, 4 patients underwent RT. Adequate SC collection was reached in 11/15 patients (median number of CD 34+ve cells 2.4±10^6/kg, range 1.1-3.2×10^6/kg). Nine out eleven patients underwent ASCT while 2 patients refused. After the induction therapy 9/15 patients (60%) achieved CR, 3/15 (20%) achieved PR and 3/15 (20%) were considered no responder. After a median follow-up of 20 months (range 3-30 months), 15/18 patients are alive (83%) and 3/18 died due to lymphoma; 3/18 patients are still not evaluable for remission status because in treatment with induction therapy; 12/15 patients (80%) are alive in CR and 3/15 (20%) patients are alive in PR. During HD-MACHOP therapy, grade III-IV WHO anemia, thrombocytopenia and neutropenia were registered in 6/18, 5/18 and 14/18, respectively. One patient developed grade IV pneumonia infection after the third course; four patients necessitated red blood cells and one patient platelets transfusion. Extra-hematological toxicity consisted of grade IV mucositis in 6 patients. Conclusion: These preliminary results underline the feasibility and the efficacy of this intensive front line therapeutic program and stimulate further patients accrual.

PO365
USE OF THERAPY WITH RITUXIMAB PLUS INTERFERON-α IN RELAPSED AND REFRACTORY DIFFUSE LARGE B-CELL NON-HODGKIN’S LYMPHOMA

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Rituximab is a chimeric anti-CD20 monoclonal antibody containing human IgG1 and κ constant regions with murine variable regions. The efficacy and safety of rituximab have been demonstrated in patients with relapsed or refractory low-grade, and follicular non-Hodgkin’s lymphoma (NHL) in several clinical trials. Previous experience with rituximab in patients with diffuse large B-cell lymphoma (DLCL) is limited, but a significant activity of rituximab was demonstrated also in this subset of patients. Interferon-α has long been known to be active in low-grade NHL, and recently it has been shown that interferon-α is an active drug also in DLCL. Based on these data and on our previous experiences of a phase II study of rituximab after priming with interferon-α in patients with low-grade/follicular B-cell lymphoma, we treated three cases with relapsed and refractory DLCL with rituximab in combination with interferon-α and we report here the results of this treatment. The patients were 45-, 37- and 67-year-old males, respectively, and their stage disease was IIa (one case) and IVa (two cases). All the patients were firstly treated with a third-generation chemotherapy regimen (Promace-CytaBOM) plus involved field radiation therapy, and a complete remission (CR) was achieved. Unfortunately, the lymphomas relapsed in a time variable from six months to four years. One case had a leukemic evolution. A therapy with interferon-α was then started in all cases at a dose of 1.5 MU/day subcutaneously for five out every seven days for the first week and then at a dose of 3 MU/day during the second week. At day 15 they received the first rituximab injection at 375 mg², which was repeated at days 22, 29 and 36. Interferon-α doses were maintained at 3 MU/day during the third week and then increased to 6 MU/day during the fourth and fifth weeks. After this course of rituximab plus interferon-α all the patients obtained a complete remission. The lymphonodes or bone marrow involvement disappeared. In one case was also achieved a complete molecular response. The therapy with rituximab in combination with interferon-α was well tolerated and there were only few episodes of grade 3 neutropenia. This report shows that the use of interferon-α in combination with rituximab was effective and well tolerated in aggressive lymphomas and that represents a useful aid to manage the relapsed disease.

PO366
CORRELATION BETWEEN HEPATITIS C VIRUS INFECTION AND B-CELL NON-HODGKIN’S LYMPHOMA IN DIFFERENT GEOGRAPHICAL AREAS

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In order to study the role of HCV infection in B-NHL we evaluated consecutive, previously untreated patients affected by B-NHL, diagnosed according to REAL classification in Piacenza (306 patients, Northern Italy) and in Reggio Calabria (111 patients, Southern Italy). Antibodies against HCV were detected in 135 among 417 patients (33%) by ELISA assay; in all but one the data matched with PCR analysis. Both in the North and in the South this prevalence was significantly higher than matched control population (p<0.001). Nevertheless, we observed an higher percentage of HCV positive lymphomatous patients in the North (35% versus 24% in the South). Hystological subtypes were differently distributed between HCV+ and negative patients, although not statistical significant (p=0.08): immunocytoma was more represented in HCV positives (adjusted residual +2.5), while MALT lymphoma was less (a.r.–1.9) as well as anaplastic B cell lymphoma (a.r.–1.9). However, separate analysis revealed that in Piacenza hystological differences between HCV+ and HCV negative patients reached statistical significance (p=0.02). Patients with HCV infection were older (65.8±12.9 vs. 61.6±14.2 years, p=0.001) and more frequently women (86 of 135, p<0.001). Extraxonal localization was found in 258 patient (62%). The spleen was the most involved extranodal site (77 patients, 30%), the stomach was the second (49 cases), the dermis the third (25 cases). Among extranodal localizations only orbit differed among HCV+ (6 cases) and HCV negative patients (4 cases). In the North orbit was more involved than in the South (3% versus 1%). We did not find an increase chemotherapy related hepatic toxicity in HCV+ patients, with the exception of patients who underwent methotrexate containing regimens. Therefore in our study HCV+ lymphomatous patients were safely managed with CEP regimen: only one toxicity among 44 cycles. Moreover, among 7 patients who experienced hepatic toxicity by methotrexate, six were able to ultimate CEP administration. Both in the North and in the South overall survival and event free survival (Kaplan Mayer, log rank test and Cox‘ regression) were not significantly influenced by HCV. So we were able to confirm a significant prevalence of HCV infection in B-NHL in different geographical areas, with the same characteristics concerning age and sex. On the other side we had to register some differences between North an South Italy such as the amount of prevalence, hystological type and extranodal localization.
ROLE OF LOW DOSE 2-CDA IN THE TREATMENT OF INDOLENT LYMPHOPROLIFERATIVE DISORDERS

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Cladribine (2-Cda) is a purine analogue active against dividing and resting lymphocytes thanks to its capacity to inhibit both DNA synthesis and repair. This drug could play an important role in the treatment of low grade NHL because most cells in these disorders are in the resting phase. Several clinical studies have shown 2-Cda’s activity in numerous indolent lymphoproliferative disorders such as HCL, CLL, lymphoplasmocytic lymphoma (LCP), Waldenstrom’s macroglobulinemia (WM), cutaneous T-cell lymphoma. In patients affected by WM, three cycles of 2-Cda at a dose of 0.1 mg/kg/die or 0.12 mg/kg/die, respectively for 7 or 5 consecutive days, resulted effective. Overall response rates of 50-85% in untreated patients and of 35-50% in previously treated ones were observed. With the aim to evaluate the efficacy and toxicity of the 2-Cda at a lower dose-intensity, 15 patients with indolent NHL (6 with WM, 9 with LCP) were treated with the drug infused as bolus intravenously at a dose of 6 mg/m² once a week for a total of six courses. In this series, median age was 63 (range 48-77 years). Patients were 5 females and 10 males; 8 patients were previously treated (3 with clorambucil or/cyclophosphamide orally; 3 with CVP schedule; 1 with FLIDA schedule; 1 with Rituximab). Splenomegaly was present in 10 pts. Lymphoadenophaties in 4. The median level of monoclonal IgM (n=10) was 377 (range 253-751 U/l). 14/15 patients were evaluable for response. One patient with lymphoplasmocytic NHL (1/14=7%) achieved a complete response and 2 (1 WM; 1 LCP) a partial response. One patient with lymphoplasmocytic depletion. Fifteen patients (60%) were treated with the combination between chemotherapy and radiotherapy and 10 were treated only with chemotherapy. The FDG-PET and CT were performed in 18 patients during follow-up or at the end of therapy to evaluate the remission and in 7 cases they were performed because of a doubt of relapse during follow-up. Results show that the schedule employed was able to stabilise the disease, with a good compliance and without any important toxicity.
**PO369**
PREVALENCE OF TRANSFUSION-TRANSMITTED VIRUS IN PATIENTS WITH LYMPHOMA

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Transfusion transmitted virus (TTV) was isolated in 1997 from the serum of a patient with post-transfusion hepatitis. TTV is a single stranded, circular DNA virus with a number of characteristics typical of animal circoviruses. The role of TTV in the pathogenesis of cryptogenic hepatitis seems negligible. However, its role in causing diseases has not been completely ruled out. We have looked for the presence of TTV DNA in serum samples of 76 untreated and previously treated advanced stage LG-NHL patients and of 30 healthy controls, by hemi-nested polymerase chain reaction (PCR). The patients were studied at the time of diagnosis and none had been previously transfused. The age of patients ranged from 14 to 80 years (median 46). We found the presence of TTV DNA in the serum of 26.6% of patients with NHL (12/45) and in the serum of 32% of patients with HD (10/31). By contrast, we found the viral DNA in only 4 of 30 healthy controls (13%). These preliminary data do not allow any reasonable statistical analysis due to the small number of patients and healthy controls that we have tested so far. Nevertheless, we think that it is interesting to report the apparent increased prevalence of TTV infection in patients suffering from lymphomas. This is keeping with the increased prevalence of HCV infection observed in NHL and with the increased prevalence of HGV infection in both HD and NHL.

**PO370**
LOW-GRADE NON-HODGKIN’S LYMPHOMAS IN THE ELDERLY: IMPACT OF A LOW-DOSE FLUDARABINE-BASED COMBINATION REGIMEN (mini-FLEC)

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The clinical course of elderly patients with low-grade non-Hodgkin’s lymphoma (LG-NHL) is often fluctuating and a therapeutic approach may become necessary in patients with active disease or progressive disease. Fludarabine (FLU) in association with mitoxantrone and/or cyclophosamide (CY) induces high response rates (40-94%) in LG-NHL patients even though a high incidence of documented infections is often reported. We have previously shown the effectiveness and a mild toxicity of a low dose FLU-based regimen (FLEC) including epirubicin (EPI) and CY, in LG-NHL patients. In the present report we wanted to evaluate the efficacy and toxicity of FLEC regimen further reduced in terms of EPI and CY doses (mini-FLEC): EPI 30 mg/m² i.v. on day one, FLU 15 mg/m²/day (max 25 mg) i.v. from day 1-4 and CY 200 mg/m²/day i.v. from day 1-4 administered to a group of 20 untreated and previously treated advanced stage LG-NHL elderly patients. The aim of the study was the reduction of therapy-related toxicity without affecting the reported good response rate. All 20 patients were evaluable for response with 30% of them achieving complete remission (CR) and 55% partial remission (PR) (overall response 85%). Ten patients have relapsed and 8 have died. Characteristics of all 20 patients enrolled in the study and response rate are summarized in the following table.

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Mini-FLEC was very well tolerated and most cases were able to carry out the therapy without hospitalization experiencing mild and transient side effects (mainly short lasting FUO and neutropenia). No patients died of infectious complications. In conclusion, despite a relatively small number of cases, Mini-FLEC regimen seems to be an effective and safe treatment for advanced treatment-requiring elderly LG-NHL patients.

**PO371**
CASTLEMAN’S DISEASE, MIXED VARIANT, OF THE MEDIASTINUM. A CASE REPORT

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Castleman’s disease (CD), is a rare entity of unknown and controversial pathogenesis and etiology, characterized by lymphadenopathy with or without symptoms. Histologically it is an example of the so-called atypical lymphoproliferative disorders; three histologic variants (hyaline, plasma-cell and mixed) and two clinical types (localized and multicentric) have been described. We reported a case of thoracic localized, mixed variant, CD. Our patient is an asymptomatic 44-year old woman with a three-month history of mild persistent microcytic anemia, thrombocytosis, elevated erythrocyte sedimentation rate (ESR) and polyclonal hyperγ-globulinemia detected at a routine check. Physical examination was unremarkable. Chest and Thoracic tomography (CT) revealed a large solitary well-circumscribed mass located in the left anterior superior mediastinum. Bone marrow aspiration and biopsy showed no lymphomatous or neoplastic infiltration. Transthoracic fine needle aspiration cytology (FNAC) was non-diagnostic but the mass was thought to be a possible thymoma; metastatic carcinoma and germ cell tumor were excluded. Left thoracotomy was performed and the...
PO372
IDARUBICIN, CISPLATIN, CYTARABINE, DEXAMETHASONE, (IPAD) AS SALVAGE/CONSOLIDATION THERAPY IN NON-HODGKIN’S LYMPHOMA, PRELIMINARY DATA

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The efficacy of a combination therapy including Cisplatin, Cytarabine (ARA-C) and dexamethasone (DHAP) as salvage therapy in refractory non-Hodgkin’s lymphoma (NHL) is well-known from 1988, as is the possibility of obtaining an adequate peripheral blood stem cell mobilization and harvest. The combination of Idarubicin and Cisplatin resulted an active and well tolerated therapy (Caracciolo et al. Leuk Lymphoma 1997) for elderly patients (pts) with NHL. To evaluate the feasibility and tolerability of the IPAD regimen as salvage therapy for pts with refractory NHL, heavily pretreated, or as consolidation therapy we treated 10 pts (various histology) with this schedule: Idarubicin 10 mg/m² d.1, Cisplatin 40 mg/m² d.1, ARA-C 1 g/m²/12h d.2, Dexamethasone 20 mg d. 1-2-3. It was administered: as consolidation therapy to 3/10 pts who are still in CR after 2, 6, 29 months of follow up; as salvage therapy (at least 3 cycles) to 7/10 pts, 2 of whom obtained CR but relapsed after 4 and 5 months. Median follow up is 10 months. Grade 4 WHO hematologic toxicity was observed in 4/10 pts, grade IV liver toxicity, jaundice, in 1/10 pts. We also treated 15 pts with an intensified schedule: idarubicin 10 mg/m² d.1-3, Cisplatin 50 mg/m² d.1-2, ARA-C 2 g/m²/12h d.3, dexamethasone 20 mg d. 1-2-3. It was administered: as consolidation therapy to 2/15 pts still in CR after 19 and 24 months of follow up; as salvage therapy to 13/15 pts. Of this group 6/13 pts achieved a clinical response: 1/6 pt is still in CR after a follow up of 43 months; 5/6 pts achieved PR but 3/5 pts experienced progression after 6, 11, 38 months. Median follow up is 13 months. We observed grade 4 hematologic toxicity in all cases but only sporadic grade 1 renal, liver and GUT toxicities. No death treatment related was documented. Adequate mobilization and collection of PBSC is allowed after the IPAD regimen. These data, mostly obtained in a group of refractory NHL highly pretreated, are not sufficient to evaluate the real efficacy of the IPAD regimen but stimulate a study on patients refractory to the first line chemotherapy.

PO373
SEQUENTIAL THERAPY WITH RITUXIMAB GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IN REFRACTORY HIGH-GRADE NON-HODGKIN’S LYMPHOMA

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Background. Rituximab showed a therapeutic activity in NHL both in monotherapy and in association with conventional therapy. Cytolytic activity of this mAb is principally due to its binding to CD20 antigen with successive Fc-mediated activation of host cytotoxic cells. With the aim to potentiate the activity of these cells and of inducing the sensitzation to lymphomatous clone, we evaluated the clinical efficacy with neo-adjuvant aim of GM-CSF, a specific growth factor for monocyte-macrophage cells, granulocytes and dendritic cells. Case report: An 67-years-old patient with HCV-related hepatitis and diagnosis of aggressive CD20+ NHL at stage IIIs, intermediate-high I.P.I. score and P.S.=80%. After unsuccessful treatment for severe neurological and hematological toxicities and resistance to three different lines of chemotherapy (ProMACE-Cyta-BOM, CNP, VNCOP-B), we decided to use Rituximab associated to GM-CSF in the following schedule: GM-CSF 1 fl s.c. for 3 days followed by Rituximab at 375 mg/m² for 9 weekly infusions. Results. Complete remission at 7 months follow-up. Discussion. This case is the first described in literature of sequential use of GM-CSF and Rituximab for the treatment of indolent or aggressive NHL refractory or relapsed. This therapeutic approach is based on the following considerations: use of Rituximab to identify the B-cells compartment, where the neoplastic clone is present, to enhance the cytotoxic pathways of the host and the mobilization of antigens (neo-Ags) from eventual chimeric proteins of the neoplastic cells; use of GM-CSF to expand the monocyte-macrophage compartment, which are the major protagonists of the immune cytotoxic response mediated by Rituximab; to give a growth factor to type I dendritic cells, when they differ from CD34+ and CD14+ precursors and they can recognize and internalize neo-Ags, so trying to sensitize the immune system. Conclusions. The drugs’ safety and the clinical response let us extend the use of this treatment also to high-grade NHL: the good results we achieved induce us to a prudent optimism, because the follow up is short.

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The patient, after the surgical resection, was treated with prednisone 1 mg/kg/die for 1 month. After a 6, 12, 24, 36-months follow-up, the laboratory tests are normal and neither symptoms nor progression of disease were reported. This case confirmes that: 1) mediastinal localized CD is a rare disorder (representing only about 1% of all mediastinal lesions) with a favorable clinical course. The recommended primary treatment is a complete surgical resection. Typical laboratory abnormalities (such as anemia, hyper-γ-globulinemia, elevated ESR, etc) usually resolve within 30-60 day after surgical resection. Close and long term follow-up is necessary to detect local recurrence and malignant sequelae; 2) CD should always be included in the list of differential diagnosis of mediastinal masses which are mostly malignant tumors (such as lymphomas, thymomas or metastases of lung cancer); 3) the role of CT guided FNAC in the diagnosis of mediastinal epithelial neoplasms is well established; however, its use for the diagnosis of mediastinal lymphoproliferative disorders has been somewhat limited (CD can be confused with other mediastinal lesions especially lymphomas or thymomas); in fact the aspiration smears are non specific, showing only an abundance of lymphocytes; in these cases mediastinoscopy or mediastinostomy are necessary to enable a precise histological diagnosis.

Background: An 67-years-old patient with HCV-related hepatitis and diagnosis of aggressive CD20+ NHL, at stage IIIs, intermediate-high I.P.I. score and P.S.=80%. After unsuccessful treatment for severe neurological and hematological toxicities and resistance to three different lines of chemotherapy (ProMACE-Cyta-BOM, CNP, VNCOP-B), we decided to use Rituximab associated to GM-CSF in the following schedule: GM-CSF 1 fl s.c. for 3 days followed by Rituximab at 375 mg/m² for 9 weekly infusions. Results. Complete remission at 7 months follow-up. Discussion. This case is the first described in literature of sequential use of GM-CSF and Rituximab for the treatment of indolent or aggressive NHL refractory or relapsed. This therapeutic approach is based on the following considerations: use of Rituximab to identify the B-cells compartment, where the neoplastic clone is present, to enhance the cytotoxic pathways of the host and the mobilization of antigens (neo-Ags) from eventual chimeric proteins of the neoplastic cells; use of GM-CSF to expand the monocyte-macrophage compartment, which are the major protagonists of the immune cytotoxic response mediated by Rituximab; to give a growth factor to type I dendritic cells, when they differ from CD34+ and CD14+ precursors and they can recognize and internalize neo-Ags, so trying to sensitize the immune system. Conclusions. The drugs’ safety and the clinical response let us extend the use of this treatment also to high-grade NHL: the good results we achieved induce us to a prudent optimism, because the follow up is short.

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A monoclonal gammopathy was present in 21 (10%) patients while in one case no cryoglobulins were detectable. Three MALT lymphomas (1%) and one chronic lymphocytic leukemia were found. Low-grade non-Hodgkin’s lymphoma (NHL) was found in 10 patients (4.7%), nine of them showed type II MC, while in one case no cryoglobulins were detectable. Among these patients, 30 (41%) showed cryoglobulinemic syndrome with purpura of the lower extremities (72%), weakness (73%) and arthralgias (68%). Peripheric neuropathy was found in 36 cases (17%), Raynaud phenomenon in 35 (17%), and sicca syndrome in 13 (6%). Elevated levels of rheumatoid factor were found in 56 (27%), low complement component four levels in 29 (14%). The biochemical evidence (high rheumatoid factor) of mixed cryoglobulinemia (MC) was observed a mean of 7 years before the clinical manifestation of the disease in 10 patients (5%). Membranoproliferative cryoglobulinemic glomerulonephritis was found in 3 cases (1.4%). Low-grade non-Hodgkin’s lymphoma (NHL) was found in 10 patients (4.7%), nine of them showed type II MC, while in one case no cryoglobulins were detectable. Three MALT lymphoma of the stomach (1.4%), two diffuse large cell lymphoma (1%), and one chronic lymphocytic leukemia were found. A monoclonal gammopathy was present in 21 (10%) patients (IgG in 12 and IgM in 9), in one case a IgGx plasma cell myeloma was found. Autoimmune thyroiditis was observed in 3 patients (1.4%), porphyria cutanea tarda in 1 and lichen ruber planus in 3 (1.4%). Elevated anti-nuclear antibodies were present in 11 patients (5.2%), anti-mitochondrial in 3 (1.4%), and anti-smooth muscle in 16 (7.6%). Conclusion: Extra hepatic manifestations are frequently observed in HCV patients. The most frequent hematological abnormalities include mixed cryoglobulinemia and monoclonal gammopathy. However, despite the widespread use of these techniques, there has been reluctance to apply them to the spleen. The aims of this study were to evaluate the efficacy and safety of UG-FNB and to summarize our experience with US-guided biopsies in the spleen performed in 150 patients and to report the technique, precaution, results and complications. One hundred and fifty patients underwent percutaneous splenic biopsies at our Institution; conditions necessary to do the biopsy were: normal thromboplastin time, prothrombine activity more than 50% and platelet count higher than 70×10^10/L. The splenic biopsies were performed with sonographic localization, access routes were selected to avoid colon, pancreas, kidney, lung and pleura. All focal lesions were checked initially with a 22-gauge needle for aspiration cytology and tissue core biopsy was done only in lesions suspected to be of lymphomatous nature at rapid cyto logic stain. In patients without focal lesions, splenic biopsies were performed either with 22-gauge needle for aspiration cytology and 21-gauge Surecut for tissue core histology. In three patients with abscesses, drainage catheters were inserted under US-guidance. Diagnostic tissue was obtained by US-guide biopsy of the spleen in 147 of the 150 patients (98%). Forty-two patients showed malignant disease: 33 non-Hodgkin’s lymphoma, 1 Hodgkin’s disease, 7 metastatic carcinoma, 1 sarcoma. One hundred and eight patients showed non malignant disease: cysts 10, abscesses 7, tuberculosis 8, granulomatosis 5, normal splenic tissue in the other patients (biopsies performed as a staging or restaging procedure in patients with a previously diagnosed lymphoma). Only two patients showed subcapsular hematoma which resolved spontaneously. Our results suggest that carefully performed biopsy and drainage techniques appear to be safe and may conserve the spleen by avoiding splenectomy.

PO376 FLUDARABINE, IDARUBICIN AND PREDNISONE (FLIDA) AS SALVAGE TREATMENT IN ADVANCED INDOLENT LYMPHOMAS

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The optimal management of indolent lymphomas is still controversial. Several papers showed that aggressive therapies may induce high complete response rates but their effect on overall survival is still not clarified. Fludarabine and Idarubicin are very effective drugs in the treatment of these disorders. Our previous experience with FLUDA regimen (fludarabine 25 mg/m^2/die intravenously on days 1,2,3; idarubicin 10 mg/m^2/die on day 1 and prednisone 150 mg/day orally on days 1-5, every 28 days for three to six cycles) in 21 untreated patients with indolent NHL demonstrated the efficacy and safety of this schedule. In this group Overall response was 95% (47.6% CR; 46.7% PR); after a median follow-up of 24 months (range 2-76), the failure-free survival was 43%. The same schedule was used as salvage therapy in 16 patients with advanced relapsed or refractory indolent lymphoma. In this series median age was 64 years (range 43-77); 14 males and two females; the median LDH was 372 U/L (range 125-700); 2 had stage III and the remaining 14 had stage IV (bone marrow involvement). 14/16 patients were evaluable for response; 3/14 obtained a CR (21%), 6/14 a PR (43%) with an overall response rate of 64%. The median duration of response was 7 months. At a median follow-up of 12 months FFS was
Analysis detected PML-RAR with Auer rods; cytogenetics showed t(15;17) and molecular examination revealed the presence of atypical promyelocytes of the same VICED regimen. After 13 months, the patient in October 1994 and achieved a second remission after six courses of vincristine, cyclophosphamide, idarubicin, etoposide and deflazacort) given every three weeks. The patient relapsed and an intra splenic hypodense mass. He obtained complete remission after ATRA therapy while NHL did not respond to the CEOP protocol. She died of lymphoma progression after a few months. The majority of NHL at presentation show non-random chromosomal abnormalities; additional genetic changes may play a role in disease progression. We describe a patient whose complex karyotype at relapse is a rare summation of unfavorable genetic events. A 57 year old woman was diagnosed as NHL in December 1994. Circulating lymphoma cells were B-lineage CD5+, with light chain restriction. Immunoglobulin (Ig) gene molecular analysis documented the presence of clonal cells in peripheral blood, bone marrow and lymph nodes; PISH analysis of bone marrow smears showed trisomy 12. Chemotherapy given is summarised in the following table:

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<td>PR</td>
<td>0</td>
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<td>PR</td>
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In December 1998 her clinical condition rapidly deteriorated, and a bone marrow aspirate revealed monomorphic large undifferentiated blasts whose immunophenotype was unmodified with respect to diagnosis. Molecular analysis showed the presence of c-myc rearrangement, while the Ig heavy chain rearrangement was no longer detectable. Bone marrow cytogenetics showed a complex karyotype: 47, XX, t(8;14) (q24;q32), del(11)(q13), +12, der(13)t(13;22)(q10;q10)x2, der(20)t(13;11)(q31;q23) +17. Circulating lymphoma cells were B-lineage CD5+, with light chain restriction. Immunoglobulin (Ig) gene molecular analysis documented the presence of clonal cells in peripheral blood, bone marrow and lymph nodes; PISH analysis of bone marrow smears showed trisomy 12. Chemotherapy given is summarised in the following table:

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PO379
INTRAVASCULAR LYMPHOMATOSIS WITH PREDOMINANT NEUROLOGICAL INVOLVEMENT, DIAGNOSED IN VIVO BY BONE NEEDLE BIOPSY

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Intravascular lymphomatosis (IL) is a peculiar variant of large B-cell lymphoma characterized by almost exclusively intravascular proliferation of neoplastic cells, and with an unexplained singular propensity to cerebral localization. IL is a multorgan disease (skin, lung, kidney and liver are commonly involved) with several clinical symptoms and laboratory alterations. The prognosis of IL is very poor even after chemotherapy and the diagnosis is frequently made post-mortem. We describe a patient whose first manifestations were neurological and the diagnosis was made on core biopsy of bone marrow when the patient was alive. In April 2000, a 50 year old man developed transient right facial paresis of central type; two weeks later he suffered from a grand-mal fit, was somnolent, quadriparetic (left>right) with facial paresis of central type; two weeks later he suffered from several clinical symptoms and laboratory alterations. The suspicion of an infective disease had been removed, diagnostic work-up addressed the hypothesis of a vasculitic disorder with prevalent neurological presentation. A cerebral biopsy was not considered because of the critical condition of the patient. The bone marrow biopsy, whose indication had been suggested by pancytopenia, proved to be diagnostic.

PO380
FLUDARABINE IN COMBINATION AS SALVAGE TREATMENT AND AS FRONT-LINE THERAPY IN LOW-GRADE NON-HODGKIN’S LYMPHOMA

Department of Hematology, S. Martino Hospital, Genoa

Fludarabine (FLU) alone or in combination has been reported to be effective in LGL. But granulocytopenia and infection occurred. In our first study, FLU was combined with cyclophosphamide (CY) and with CY plus mitoxantrone (MITO). Fifty-three successive pts with recurrent LGL (R.E.A.L. classification) entered studies. Patients. Median age 56 years (range 35 to 75); stage II 5 pts, stage III-IV 48 pts; relapse after CR 20 pts, PR in progression 21 pts, NR 6 pts, PD 6 pts. Twenty-two patients received FLU/CY treatment (FLU 25 mg/m² days 1 to 3, CY 300 mg/m² days 1 to 3), and 31 patients FLU/CY/MITO treatment (FLU 25 mg/m² days 1 to 3, CY 300 mg/m² days 1 to 3, MITO 10 mg/m² day 1). Courses were given every 4 weeks for a maximum of 6 courses. Patients received antibiotic prophylaxis throughout treatment and growth factor (G-CSF) when grade III granulocytopenia (WHO) occurred. Thirty-one pts achieved CR (58%) and 16 PR (30%), with an overall response rate of 88%. Response was similar in both groups. Few febrile episodes occurred without infection. The antibiotic prophylaxis with G-CSF support seemed to reduce treatment-related infection. Following these observations a front-line study using FLU/CY/MITO for 6 courses in 60 LGL untreated patients was begun. Patients. Median age 60 years (range 36 to 72); stage II 2 pts, stage III-IV 58 pts; BM involvement 38 pts. Fifty pts are evaluable for response. Thirty-two pts achieved CR (64%) and 14 PR (28%), with an overall response rate of 92%. Myelosuppression was the most evident toxic effect and grade III-IV leukopenia occurred in 46% of treated patients. Mild anemia and thrombocytopenia (grade I-II, WHO) occurred in 24% and in 25% of pts, respectively. One infection was observed and 7 pts experienced fever without infection. The 3-yr probability of PFS is 81%. These studies confirm that FLU in combination provides an effective and safe treatment for LGL.
The pathological diagnosis of lymphoma can require surgical laparotomy when are present only deeply located abdominal adenopathies. Imaging guided biopsy was recently proposed in these cases with the aim to avoid surgical procedures; the reported results are encouraging (overall accuracy of 64-90%). Nonetheless, information about clinical and technical factors affecting overall accuracy in these cases are scanty. In our series, 191 patients underwent Ultrasound guided fine needle biopsy of deeply located abdominal adenopathies: among these, 104 had a previous diagnosis of cancer and 87 were at onset of their disease. Cytological sampling was performed in 189 cases and hystological specimens using fine needles (less than one millimeter of outer diameter) were taken in 62 cases. Therefore, 2 patients received only hystological biopsy and 60 patients received double sampling (cytology and hystology). Among our patients, 82 suffered from lymphoma (7 cases of Hodgkin’s disease, 47 of aggressive Non Hodgkin Lymphoma and 28 of indolent lymphoma). The obtained results were analyzed according to type of sampling, subtype of lymphoma, timing of the procedure (first diagnosis or diagnosis of recurrence). In the whole group we obtained an overall accuracy of 66,1% for hystology, 77,7% for cytology and 83,3% for double sampling. In the patients with lymphoma, we observed an overall accuracy of 71,2% for cytology, 65,9% for histology and 76,1% for double sampling. The best results were obtained in the group of aggressive lymphomas, particularly for cytology (84,4%). Moreover, the overall accuracy was higher in patients with previously known diagnosis in comparison with those with no previous diagnosis. In 26/28 cases the employment of immunohistochemical staining allowed the correct subtyping of non Hodgkin’s lymphoma according to the current classifications (Working Formulation and REAL). Conversely, it is important to note that in 5/7 cases of Hodgkin’s disease insufficient material was obtained both with cytology and histology. Therefore, we conclude that Ultrasound guided fine needle biopsy with cytological and histological sampling (with the addition of immunohistochemical techniques) is an efficacious technique for the pathological diagnosis of deeply located abdominal adenopathies suspected for lymphoma and adequate subtyping can be obtained in most cases. Best results were obtained in aggressive lymphomas, when double sampling is performed and when a previously known pathological diagnosis is available. However, in some patients (particularly in those with Hodgkin’s disease) a surgical diagnostic laparotomy is needed to obtain a definite pathological diagnosis.
E. Morra, A.M. Cafro, P.L. Oreste,* G. Muti, M. Anghilieri, M. Montillo

HEART TRANSPLANT
PERIPHERAL T CELL LYMPHOMA INVOLVING TRANSPLANTED ORGAN AFTER NEOPLASIA DIAGNOSIS

This method may be helpful in the differential diagnosis of lymphadenopathies.

The risk of developing lymphoproliferative disorders in patients receiving immunosuppressive therapy after organ transplantation, is increased 20 to 120 fold comparing with immunocompetent population. About 90% of PTLD are malignancies deriving from B-cell lineage, while 10% derive from T-cell lineage. Hodgkin's disease is rare. Extralocalizations are very frequent especially in the gastrointestinal tract. Localization in the transplant- 
ed organ has been described: frequently in lung and liver, rare in the heart. Case report. A 58-year-old man who underwent orthotopic heart transplantation for dilated cardiomyopathy. Sepsis from Staphilococcus and Enterococcus, HSV and CMV infections was detected in the post-transplantation phase. In conditioning immunosuppressive regimen anti-lymphocyte serum (SAL) Cyclosporin A (CSA), azathioprine and steroids were employed, and then in maintenance phase CSA and low doses of steroid were administered. Anemia and thrombocytopenia appeared 15 months after transplantation have been initially correlated with immunosuppressive therapy. A lymphnode biopsy was performed when inguinal node > 2 cm size was detected. Histology showed features of a medium-size cell non Hodgkin's lymphoma of T cell origin (CD45RO+, CD3+, CD20-, CD79α+, MIB 80%), described by the REAL Classification as peripheral T medium-size cell lymphoma. No infiltration was detected in bone marrow biopsy. Total body computed tomography scan showed lung nodules, especially in the right lung and hepaticomegaly. At admission the patient was febrile presenting inguinal lymphnodes and Kaposi-like skin lesions in abdomen and lower limbs. Serology for EBV, CMV, HTLV I-II were negative. HTLV I-II PCR-determination were negative also. Patient poor conditions did not allow chemotherapy administration, but only reduction of immunosuppressive regimen; because the fast evolution of the neoplasia the patient died 18 days after diagnosis from multiorgan failure. Many localizations of lymphoma were observed at the autopsy: lymph nodes (mediastinal, clavicular, aortic, inguinal); liver (malignantnodules), pleura, lung, heart (node in septal atrium) and ulcerated mucosal mass of the stomach (diameter 10 cm) infiltrating the wall involving the serosa and extending to the ileum. Histopathologic and immunohistochemical characteristics were identical in all sections obtained from different organs. PCR and FISH tests for EBV and HTLV I/II were also negative in malignant lymphoid cells. Conclusions. 1. PTLD report of a pleomorphic lymphoma of T cell origin is rare in the literature; 2. localization in the grafted heart is unusual; 3. severe prognosis of PTLD of T cell lineage was confirmed.

A.M. Cafro, P.L. Oreste,* G. Muti, M. Anghilieri, M. Montillo, E. Morra

Division of Hematology, Dept of Oncology: Hematology; *Service of Pathology, Niguarda Ca' Granda Hospital, Milan

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Case report.

Asymptomatic Hepatitis C Virus Positive Patients with or Without Non-Hodgkin's Lymphoma

Liver histology both in terms of grade and stage of the neoplasia the patient died 18 days after diagnosis from multiorgan failure. Many localizations of lymphoma were observed at the autopsy: lymph nodes (mediastinal, clavicular, aortic, inguinal); liver (malignant nodules), pleura, lung, heart (node in septal atrium) and ulcerated mucosal mass of the stomach (diameter 10 cm) infiltrating the wall involving the serosa and extending to the ileum. Histopathologic and immunohistochemical characteristics were identical in all sections obtained from different organs. PCR and FISH tests for EBV and HTLV I/II were also negative in malignant lymphoid cells. Conclusions. 1. PTLD report of a pleomorphic lymphoma of T cell origin is rare in the literature; 2. localization in the grafted heart is unusual; 3. severe prognosis of PTLD of T cell lineage was confirmed.

PO385
ORAL CYCLOPHOSPHAMIDE THERAPY FOR TRANSFORMED HIGH-GRADE LYMPHOMAS

R. Riccioli, S. Galimberti, G. Cervetti, R. Fazzi, F. Caraccio, M. Petroni
Hematology Division, Department of Oncology, Transplant and Advanced Technologies, University of Pisa

In a retrospective study on high-grade non-Hodgkin’s lymphomas (NHL) 148 aggressive NHL were analysed in order to evaluate the histology at the relapse by lymphonode or bone marrow biopsy. 18 cases (12%) were proved to have a low-grade lymphoma relapsed or persistent after CHOP like therapy. In 5/18 association of low and high grade lymphoma was detectable at diagnosis by bone marrow biopsy. In the remaining 13/18 no evidence of indolent lymphoma was detected at the first evaluation. Outcome of these patients was compared to that of 43 patients relapsed without changing in histology and treated by a second line therapy. Thirteen were not responders, 10 achieved a partial remission and 18 complete remission. Two were lost at the follow-up. The 18 patients relapsed with indolent subtype received daily oral cyclophosphamide (100 mg/day for 15 days every month for 6 months): 3 of them were NR, 5 CR, and 10 PR. With a median follow-up of 3 years the disease free survival rate was zero in both arms; patients with high-grade disease were refractory or relapsed after a median of 3-4 months, whereas the cases with low-grade showed a long lasting partial remission stable for a long period. In fact the overall survival rate is 20 months and 39 months respectively (Breslow test p<0.02). We suggest that patients relapsed with low grade lymphoma were, in fact, transformed low-grade at the diagnosis and, after removing the high grade component by chemotherapy it is possible to manage these patients by conventional therapy for indolent lymphomas.

PO386
HISTOLOGICAL COMPARISON OF CHRONIC LIVER DISEASE IN ASYMPTOMATIC HEPATITIS C VIRUS POSITIVE PATIENTS WITH OR WITHOUT NON-HODGKIN’S LYMPHOMA

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Background. Patients infected with hepatitis C virus may develop B-cell non-Hodgkin’s lymphoma (B-NHL). It is still unknown whether in these patients NHL originates from the hepatic disease or from the infection itself. We have addressed this question by comparing liver histology and liver disease outcome in a cohort of asymptomatic HCV* subjects without and with NHL. Patients and Methods. We studied 55 asymptomatic HCV positive patients, 37 without and 18 with B-NHL. All patients underwent liver biopsy, that was evaluated according to Ishack’s score. Patients were followed for seven years and ALT determined every two months. The end point of the follow-up was raised ALT, which was considered as a marker of liver disease progression. Results. Liver histology both in terms of grad-
ing and staging was significantly milder in the group of B-NHL patients. During the follow up 22% of the patients had increased ALT in the group without NHL, according to the calculated Kaplan-Meier curve, while all NHL patients showed persistently normal ALT values. Conclusions. HCV positive patients affected by B-NHL do not have a more severe liver disease as compared to patients without B-NHL. This means that it is unlikely that NHL in HCV+ patients is a consequence or perturbed immune system by the hepatic disease. Rather, it seems that HCV may cause either hepatitis or NHL, occasionally both.

Conclusions.

HCV positive patients affected by B-NHL do not have a more severe liver disease as compared to patients without B-NHL. This means that it is unlikely that NHL in HCV+ patients is a consequence or perturbed immune system by the hepatic disease. Rather, it seems that HCV may cause either hepatitis or NHL, occasionally both.

Dendritic cells (DC) are professional antigen presenting cells that play a central role in the initiation and modulation of the immune response. It has been reported that chronic myeloid leukemia (CML) cells can be induced to differentiate into functional DC which retain the capability to induce autologous antitumor specific immune responses. Recently the WHO has recognised the myelodysplastic/myeloproliferative disorders (MPD/MDS) as a separate entity in the new classification of hematologic myeloid disorders. The aim of this study was therefore to compare the phenotypic and functional characteristics of DC generated in vitro from the peripheral blood mononuclear fraction of 10 MPD/MDS patients (7 CMML and 3 atypical CMML with DCs generated in a similar way from 17 CML patients in chronic phase. After 14 days of culture in the presence of GM-CSF, IL-4 and TNF-α, comparable numbers and percentages of DC were obtained in the two groups of patients. MPD/MDS pre-culture monocytes exhibited a significantly higher intensity of expression of CD14 (p=0.051) and chemokine receptors CCR5 (p=0.066) and CXCR1 (p=0.066). By contrast, MPD/MDS DC presented significantly lower intensity of expression of CD11b (p=0.040), CD11c (p=0.001), CD1a (p=0.053) and CD83 (p=0.023) molecules while no difference was observed concerning co-stimulatory antigens (CD40, CD80, CD86) and MHC class II molecules. MPD/MDS DC also showed a reduced receptor-mediated endocytosis as demonstrated by FITC-dextran uptake (p=0.018). By contrast, the ability to stimulate T cells in the allogeneic mixed leukocyte reaction (evaluated by 24 hour BRDU incorporation) was not different in the two groups of patients. Simultaneous FISH and immunophenotypic analysis demonstrated that in both groups of patients DCs were derived from the pathological clone. Taken together these findings indicate that, not only in CML but also in MPD/MDS disorders monocyte-derived DC are part of the malignant clone. Moreover, in MPD/MDS, pre-culture monocytes are in a functional state of activation which could influence DC differentiation. Though exhibiting a deficient expression of DC markers and a deficient FITC dextran uptake as observed in MDS disorders, MPD/MDS DC are still capable of inducing an apparently normal proliferation of allogeneic T cells. Further studies are warranted to clarify the reasons for these abnormal phenotypic and functional findings in both monocytes and monocyte-derived DC and to verify whether a deficient uptake function could present some problems in the pulsing of DC with specific peptides for the design of immunotherapeutic strategies.
The documentation of splenomegaly is a parameter which does deserve attention in several myeloproliferative (MP) disorders including essential thrombocytemia (ET). With the aim of evaluating in an objective and concrete manner spleen enlargement, we have planned to use extensively ultrasound scan (US) in patients affected by any type of myeloproliferative diseases. Here we report our experience testing the fitting of one parameter, spleen volume (SpV), in the definition of splenomegaly in ET affected patients. We considered 44 consecutive patients (29 female, 15 male; median age 60 years, range 17–75) with thrombocytosis (>600,000/µL) and 14 normal control subjects (CS) matched for sex, age and body surface. In order to concentrate the study on inapparent or mild splenomegaly, patients showing marked spleen enlargement at physical examination (p.e.) were excluded. Spleen longitudinal diameter and perimeter were measured using an Hitachi (EUB 525) US instrument, so that area and volume were automatically calculated. At the end of the diagnostic work up, according to the Polycytemia Vera Study Group (PVSG) criteria, 33 patients were diagnosed as Essential Thrombocytemia (ET), 4 as idiopathic myelofibrosis (IMF) and 7 as secondary thrombocytosis (ST). While p.e. revealed enlarged spleen only in 11 out of the 44 patients, spleen volume (SpV) by US scan was increased in 29. In the CS group, spleen volume ranged between 60 and 190 mL (median value 80). In the ET group, 25/33 (75%) patients had US splenomegaly (median 330 mL, range 90–700); the difference between ET and CS median spleen volumes was statistically significant (p< 0.001 ANOVA test). When we compared SpV with bone marrow (BM) histopathology features (fibrosis/dysmecayropoiesis), 28 patients tested positive for both procedures could be divided into 3 sub-groups: (i) 9 patients with mild BM abnormality had no or minor splenomegaly (median 120 mL, range 100–500); (ii) 14 patients with moderate BM abnormality had moderate splenomegaly (median 360 mL, range 80–700); (iii) 5 patients with severe BM abnormality had marked splenomegaly (median volume 500 mL, range 120–700). We may speculate that group (i) had either ET in very early phase or a non MP disorder (such as secondary thrombocytosis). Group (ii) had ET in early stage. Indeed in the IMF group 4/4 patients had US splenomegaly in the same range as group (iii) (median 490 mL, range 430–740). In conclusion, US scan was an accurate, reliable and easy tool to identify silent (non palpable) splenomegaly in patients with thrombocytosis. US measurement of SpV is more sensitive than single linear measurement of one dimension. These conclusions seem to apply even in other MP disorders now in study (PV, IMF).
PO390
PROLIFERATIVE ACTIVITY OF HEMOPOIETIC ERYTHROID PRECURSORS AND ERYTHROPOIETIN LEVELS IN SUBJECTS WITH PHYSIOLOGICAL AND PATHOLOGICAL RESPONSE (CHRONIC MOUNTAIN SICKNESS) TO HIGH ALTITUDE STAY

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Chronic mountain sickness (CMS) develops in subjects living at high altitude. It is characterized by an over-response of physiologic and adaptative mechanisms to high altitude with the appearance of a severe erythrocytosis. Therefore CMS represents a study model for disorders due to a strong stimulation of erythropoiesis. During an international scientific expedition recently accomplished in Cerro de Pasco, Peru (4330 m o.s.l.) focused on hematologic, cardio-pulmonary and circulatory parameters related to the excessive erythrocytosis, we examined peripheral blood hemopoietic erythrocytic precursors and erythropoietin (Epo) levels in: (A) subjects continuously living above 4000 m o.s.l. and affected with CMS, (B) subjects living in the same place without CMS, (C) subjects of the same ethnic group living at sea level (Lima, Perù, 0-60 m o.s.l.); all subjects were males, mononuclear cells obtained from peripheral blood after Ficoll separation, and serum for Epo detection were frozen in liquid nitrogen vapors and examined in our laboratory, in Italy. The cells, after an acridine orange-based vitality test, were cultured in methylcellulose (Methocult, Stem-Cell, Canada) in the presence and in the absence of erythropoietin. Optical microscopy analysis of hemopoietic precursors (BFU-E) has been carried out after 14 days culture. Epo detection was performed with an EUSA assay (Quantikine RD System). The number of BFU-E did not show significant differences in the three groups studied (P: 0.263), particularly in Epo-independent proliferation (autonomous BFU-E) (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>n.</th>
<th>Median age yrs (range)</th>
<th>Ht%±SD</th>
<th>BFU E*</th>
<th>autonomous BFU-E**</th>
<th>Epo miU/mL±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15 41 (29-51)</td>
<td>65±7</td>
<td>1.7±0.8</td>
<td>0.26±0.1</td>
<td>31±9.7</td>
</tr>
<tr>
<td>B</td>
<td>15 35 (22-54)</td>
<td>54±5</td>
<td>1.6±0.4</td>
<td>0.2±0.2</td>
<td>11.1±1.3</td>
</tr>
<tr>
<td>C</td>
<td>15 32 (22-43)</td>
<td>43±2</td>
<td>3.5±1.5</td>
<td>0.1±0.9</td>
<td>5.6±0.4</td>
</tr>
</tbody>
</table>

A: CMS subjects; B: subjects with adaptive polycythemia living in high altitude; C: Andean living at sea level; *: colonies/2x10^5 cell ± SE; SE: Standard Error; SD: Standard Deviation.

The number of progenitors did not appear proportional to Epo levels, which was higher in high altitude subjects and particularly in CMS patients, as a feedback due to the high number of RBC. These data seem to suggest a normal proliferative pattern of erythroid precursors in all the examined subjects. The strong erythrocytosis of CMS seems not to be due to hypersensitivity of precursors to the Epo, as observed in polycythemia.

PO391
MYELOFIBROSIS WITH MYELOID METAPLASIA: EVALUATION OF RECENT DIAGNOSTIC CRITERIA ON 215 PATIENTS

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A retrospective clinical study was conducted in 215 patients (131 males, 84 females, median age 65 years) with a diagnosis of myelofibrosis with myeloid metaplasia (MMM); observed consecutively from 1975 to 2000 in our centre; 206 patients were evaluable and the median follow up was 39 months (range 1-176). At the end of observation 100 patients had died and 106 were alive; median survival was 70 months. Fever, weight loss, nocturnal sweating and abdominal distress affected about 50% of patients at diagnosis; splenomegaly was present in 80% and was massive in 14% of patients. No differences at presentation have been observed in patients recently included in this study as compared to patients diagnosed before 1990. A retrospective evaluation of the diagnostic criteria used led us to divide patients into three distinct groups: 1. true MMM (67 cases) according to the Italian Criteria derived from the Italian Consensus Conference; 2. questionable but very likely MMM (60 cases) with teardrop poikilocytosis, leuko-erythroblastic blood picture, splenomegaly and diffuse bone marrow fibrosis; 3. false MMM (77 cases) diagnosed as MMM on the basis of a certain degree of fibrosis seen at bone marrow biopsy, often associated with thrombocytosis and mild splenomegaly, but lacking the remaining criteria requested for the definition of MMM. Survival curves of patients, according to new grouping, showed significant differences between group 1 and group 3, (survival at 120 months 27% versus 48%, p<0.02), but failed to reveal significant differences between group 1 and group 2 (27% versus 20%). We therefore performed a new evaluation on 127 patients of groups 1 and 2 since the group 3 turned out to be constituted by heterogeneous population including essential thrombocythemia and other subtypes of chronic myeloproliferative diseases. Median survival was 60 months; 23% of patients were younger than 55 years of age at presentation and had a median survival of 115 months versus 58 for those >55 years (p<0.002). Besides age we used haemoglobin and WBC count as prognostic factors, as recently proposed by Dupriez (Lille Scoring System for predicting survival). The median survival of the 73 low-risk patients was significantly longer (p<0.02) as compared to 39 intermediate-risk patients and 17 high risk (80 months versus 40 and 30, respectively). The median survival of 22 low-risk patients <55 years of age was 152 months. Ninety patients (60%) were treated with cytotoxic agents (mainly HU) with no difference on overall survival as compared to control. Splenectomy has been performed in 21 cases (12.5%), and was associated with a high incidence of blast transformation (28.5% compared to 9.4% in non splenectomized group). We conclude that a detailed evaluation of diagnostic criteria for MMM is mandatory not only for proper diagnosis but also for the prognostic evaluation of the disease and subsequent therapeutic decisions.
THALIDOMIDE THERAPY FOR MYELOFIBROSIS
PO393

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Myelofibrosis with myeloid metaplasia is a rare myeloproliferative disorder characterized by bone marrow fibrosis and extramedullary hematopoiesis. Recently, an increased level of the extra cellular matrix and of angiogenesis have been reported. These changes seem to be secondary to an excessive production of several cytokines such as VEGF, TGF-β and bFGF. The increased angiogenesis has suggested that the anti-angiogenic agent thalidomide could be particularly useful since it is also endowed with strong anti-cytokines properties. Therefore, we have started a study to assess the efficacy and tolerability of thalidomide in myelofibrosis in any stage of the disease. Six patients have been enrolled. The diagnosis was assessed by standard criteria, bcr-abl rearrangement (PCR) was negative in each patient. Three patients showed a stable disease, without transfusion requirement (compensated myelofibrosis), while the 3 others have become transfusion-dependent, requiring from 2 to 4 U of PRBC/month (decompensated myelofibrosis). Bone marrow biopsy was performed before therapy and repeated every 3 months while spleen ultrasonography every month. Thalidomide was administered by oral route (100 mg tablet), starting with 100 mg/daily increasing the dose to the maximum tolerated. At sixth month, no effects on Hb level, leukocyte count, and spleen size was observed in decompensated myelofibrosis, while, in compensated patients, the mean Hb level increased, the spleen size decreased (20%) and leukocyte count returned to normal level. The main side effects of the therapy were asthenia (75%), fluid retention (75%), and constipation (50%). A reduction of bone marrow cellularity was shown in the responders. In these cases, the marrow angiogenesis, evaluated as microvascular density, was reduced (from 6.0 to 4.5). On the basis of these preliminary results, thalidomide seems to have a therapeutic activity in myelofibrosis. The drug seems to be not useful in the advanced stages of the disease, when extreme fibrosis or osteosclerosis have developed. In contrast, in compensated myelofibrosis thalidomide seems to have a good efficacy, both improving hemoglobin levels, and determining spleen shrinkage.

PO394
SOLUBLE ANGIOGENIC FACTORS: IMPLICATIONS FOR CHRONIC MYELOPROLIFERATIVE DISORDERS
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Chronic myeloproliferative disorders (CMD) are characterized by varying degree of extramedullary hematopoiesis (EMH) and a high incidence of thrombotic events. The mechanisms of these complications are still not clear. Our study was prompted by the recent observation of increased bone marrow (BM) microvessel density in patients with CMD. The evaluation of this angiogenic activity, like solid tumours, might be a clue for a better understanding of the mechanism involved in the pathogenesis of EMH and thrombosis in these diseases. We have assessed the plasma and serum levels of two major angiogenic factors, Vascular Endothelial Growth factor (VEGF) and basic Fibroblast Growth Factor (bFGF) in 55 patients affected by chronic myeloproliferative disorders (CMD). This series included 25 pts with essential thrombocytemia (ET), 10 pts with chronic myelocytic leukemia (CML), 14 pts with Polycythemia Vera (PV), and 6 pts with primary Myelofibrosis (MF) and they were compared to 20 healthy control subjects. VEGF (on plasma) and sFltG (on serum) were measured in duplicate by an enzyme-linked immunosorbent assay (ELISA) method (Quantikine, R&D system, Minneapolis, USA). In all patients the plasma VEGF concentration
was significantly increased to the healthy control group (p<0.004). The highest concentrations were found in the patients with ET (178.25±125.22 pg/mL). The VEGF levels were significantly higher in CMD patients with vascular complications than those in CMD patients without complications (p<0.01). The bFGF serum levels also appeared to be significantly higher in almost all the CMD patients compared to the control group (p<0.07). A significant correlation was found between the VEGF levels and the platelet count in the ET patients and the spleen index in the CML pts. The presence of mRNA for VEGF has been described in platelets and megakaryocytes. This finding has implications for processes involving platelet and endothelial cell interactions. Previous experiments have emphasised the role of VEGF in thrombogenesis: the VEGF released by activated platelets would seem to promote endothelial activation with a subsequent switch to a predominant prothrombotic phenotype. This observation suggests that the increased plasma VEGF levels might be an important signaling molecule for thrombotic risk in CMD patients. In conclusion, in our study there is evidence of increased levels of angiogenic factors in CMD patients. Antiangiogenic therapy might be considered in patients with extramedullary hematopoiesis and/or thrombotic risk.

PO395
COMPARISON OF PERIPHERAL BLOOD AND BONE MARROW BFU-E GROWTH FOR THE DIFFERENTIAL DIAGNOSIS OF POLYCYTHEMIAS AND THROMBOCYTOSIS

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Polycythemia Vera (PV) is characterized by a clonal population of abnormal erythroid progenitors that are supersensitive to erythropoietin (Epo). This property has been utilized for laboratory differential diagnosis between PV and secondary polycythemia (SP); when cells derived from patients with PV are cloned in semisolid culture medium, erythroid colonies develop also when Epo (autonomous-BFU-E). The same diagnostic significance was observed in patients with thrombocytosis, where the BM a-BFU-E were in agreement with the diagnosis (ET or ST) in 11 cases (58%) while the PB a-BFU-E were significant in 6 cases (100%). According to these data we conclude that: 1) the presence of PB or BM autonomous BFU-E by itself is only indicative but not conclusive for PV or TE; 2) BFU-E from PB cells may be used with advantage, compared to BM cells, in the differential diagnosis of polycythemia and thrombocytosis.

Table 1.

<table>
<thead>
<tr>
<th>N. cases</th>
<th>Age yrs (range)</th>
<th>Ht% (SD)</th>
<th>Epo ml/L (SD)</th>
<th>Plt x10^9 (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV</td>
<td>60</td>
<td>62 (28-86)</td>
<td>59.44 (3.1)</td>
<td>5.2 (4.6)</td>
</tr>
<tr>
<td>SP</td>
<td>16</td>
<td>57 (26-71)</td>
<td>51 (2.8)</td>
<td>10.2 (18)</td>
</tr>
<tr>
<td>ET</td>
<td>25</td>
<td>47 (24-79)</td>
<td>45 (2.7)</td>
<td>nd</td>
</tr>
<tr>
<td>ST</td>
<td>3</td>
<td>67 (52-75)</td>
<td>44 (6.2)</td>
<td>nd</td>
</tr>
</tbody>
</table>

SP: secondary polycythemia; ST: secondary thrombocytosis; SD standard deviation.

In cases of polycythemia we cultured 37 samples from BM only, 32 from PB only and 7 from both BM and SP. In cases of thrombocytocemia 19 samples were cultured from BM and 6 from PB. Analyzing the BM cells in PV patients (37 cases) the BFU-E were 0 in 12 cases (32%) and >0 (mean 2.8±0.7) in 25 cases (68%); the BFU-E (27 cases) were 0 in 4 cases (14%) and >0 (2.9±1.4) in 23 cases (86%). In subjects with secondary polycythemia the BM BFU-E (7 cases) were 0 in 4 subjects (57%) and >0 (10±5) in 3 cases (43%); the BFU-E (12 cases) were 0 in 11 patients (92%) and >0 (2) in 1 case (8%). Therefore the BFU-E growth was diagnostic with PB cells in 87% of the polycythemia cases and only in 66% of the cases using BM cells (p<0.05). The same diagnostic significance was observed in patients with thrombocytosis, where the BM a-BFU-E were in agreement with the diagnosis (ET or ST) in 11 cases (58%) while the PB a-BFU-E were significant in 6 cases (100%). According to these data we conclude that: 1) the presence of PB or BM autonomous BFU-E by itself is only indicative but not conclusive for PV or TE; 2) BFU-E from PB cells may be used with advantage, compared to BM cells, in the differential diagnosis of polycythemia and thrombocytosis.

PO396
KIDNEY TRANSPLANTATION FOLLOWED BY DONOR BONE MARROW INFUSION IN ANIMALS (PIGS): AN EXPERIMENTAL PROTOCOL TO IMPROVE ORGAN TOLERANCE

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The presence of microchimerism, the so called bidirectional traffic of immunocompetent cells between donor and recipient and conversely, is known to ameliorate the take and survival of a solid organ transplant. To increase the microchimerism and to improve the solid organ acceptance we evaluated the possibility to infuse the donor bone marrow (BM) cells in 7 out of 16 outbred non related white large piglets submitted to heterotopic kidney transplantation (KT). The groups analyzed were as follow: Group A=3 cases, no immunosuppressive therapy (IS); Group B=6 cases, IS only (0.6 mg/kg/die oral FK 506±10 mg/kg bid oral mycophenolate mofetil: from day 0 till +30); Group C=7 cases, IS (as group B) + BM infusion at day +7 (1.25±0.73±104 kg mononuclear cells from donor iliac crest). In recipients the persistence of in vitro CFU-GM growth was considered indicative of BM viability. The hemopoietic cell cultures were performed at days: O, +7, +15, +30, +45, +60, +90. In group A the 3 animals died of acute cellular reject (ACR) between 7 and 11 days. In group B one animal survived well till day +90 (end of the study) while 5 subjects died at +17, +21, +32, +56 and + 64, (1 for peritonitis, 2 of pericarditis and 3 of pneumonitis). The survival was better in group C where 5 subjects survived till day +90 and 2 animals died, at +31 and +65 days (peri carditis and bowel obstruction). The incidence of infection-related mortality was 84% in Group B and 14% in Group C. Severe/moderate ACR were not observed in B and C animals. In group B the CFU-GM decreased progressively from 1.88±0.82 (day 0) till 15/105 cells (day 90), while in the group C they decreased only at the end of IS and then rise. In conclusion BM infusion seems to ameliorate the survival rate after solid organ transplantation. Microchimerism is under genetic/molecular evaluation.
Several cases of Familial Polycythemia have been reported since 1985, characterized by pure erythrocytosis, without leucocytosis, thrombocytosis and splenomegaly, and benign course. On the other hand, only few cases of Familial Thrombocytemia have been reported; in some of them mutations of the TPO gene were found. We describe here 7 families with 2 or more members affected by familial myeloproliferative disorders (FMD) seen in our Institution over the last 17 years. They are 17 patients, 7 males and 10 females, median age 52 years. The diagnostic workup included abdominal ultrasound, bone marrow aspirate and biopsy, karyotype and/or molecular analysis for BCR/ABL, EPO level and Hb electrophoresis. According to PVSG criteria 5 cases were classifiable as Polycythemia Vera (PV) and 12 as Essential Thrombocytemia (ET). In 4 families both cases of PV and of ET were diagnosed (mixed phenotype). In all cases thrombocytosis was present (mean 704×10^9/L). Mild leukocytosis and splenomegaly were found in 11 and 8 cases respectively. Eight patients complained symptoms due to impaired microvascular circulation; 3 underwent major vascular events (2 myocardial infarction, 1 cerebral ischemic attack). In all but 3 cases antiaggregant drugs were given; patients with PV phenotype were treated with venesection; 13 patients received chemotherapy (mainly hydroxyurea). So far, no case of plastic transformation or secondary myelofibrosis occurred. In conclusion: 1) FMD (probably not extremely rare) have to be considered in the diagnosis of chronic myeloproliferative disorders; 2) the disease phenotype can be variable, but thrombocytosis seems to be constant; this finding suggest, at least in some cases, a pathogenetic mechanism involving more than one hemopoietic lineage 3) the clinical course appears to be benign, though major vascular complications may occur; 4) therapeutical standards have to be ascertained.

Increased bone marrow angiogenesis has been demonstrated in a variety of hematologic disorders, including Polycythemia vera (PV). The neo-angiogenesis process is believed to be secondary either to the cytokine-mediated detrimental bone marrow stromal reaction or to the aberrant bone marrow microenvironment that may host excess angiogenic cytokines. Vascular endothelial factor (VEGF) and Hepatocyte growth factor (HGF), two stromal cells-derived factors, are known to be critical for vasculoangiogenesis. In this work, we determined circulating VEGF and HGF levels in patients with PV and we correlated these results with platelet count. 11 patients with PV (8 males and 3 females, mean age 60.9 years, range from 37 to 80) were included in this study. PV was defined according to Polycythemia Vera Study Group (PVSG) criteria. The duration of disease ranged between 1 year to 15 years. The patients were receiving or not receiving cytoreductive treatment. Peripheral blood VEGF and HGF levels were measured by ELISA. The median VEGF and HGF levels were significantly higher than normal controls (p<0.0001). When we correlated the VEGF and HGF concentrations with platelet count a significant correlation (r=0.85 p=0.001 and r=0.70 p=0.017 respectively) we found. Since Thrombopoietin (TPO) is a cytokine mainly derived from bone marrow stromal cells, we evaluated in 6 out of 11 studied patients the TPO levels in order to verify a correlation between VEGF, HGF and TPO. TPO concentrations were increased in patients (median value 177.5 pg/ml) compared with normal controls (median value 102 pg/ml). A significative correlation between either VEGF and TPO (p=0.006) or HGF and TPO (p=0.002) was found. These preliminary findings might suggest that neo-angiogenesis is an integral component of the bone marrow stromal reaction in PV and might provide useful prognostic information and a rationale for the therapeutic investigation of anti-angiogenic agents.

We retrospectively analyzed the records of 54 consecutive patients younger than 40 years with Essential Thrombocythemia (diagnosed according to the Polycythemia Vera Study Group criteria) followed in our department between 1990 and 2000. Median age at disease onset was 27.5 years (range 16-39), median platelet count 855×10^9/L (range 650-2190×10^9/L) and male/female ratio 2.6/1. The mean duration of follow-up was 86±42.4 months (median 97 months, range 6-136). Twenty-six percent (14/54) of patients have one (12/14) or more (2/14) cardiovascular risk factors (smoke, dyslipidemia, hypertension, diabetes mellitus). Major complications occurred in 31% (17/54) of patients (cerebrovascular accident in 2, acute myocardial infarction in 4, abortion in 8, venous thrombosis in 1 and hemorrhage in 2); none of which was fatal. Minor events (headache, erythromelalgia, paresthesias, minor bleeding) occurred in 33% (18/54) of patients whereas 46% (25/54) remained asymptomatic throughout follow-up. Forty-one of 54 patients (76%) received acetylsalicic acid and 54% (29/54) cytoreductive therapy: hydroxyurea in 7/29, busulphan in 4/29, anagrelide in 1/29, interferon in 11/29, hydroxyurea and interferon in 6/29. 79% of patients responded well to cytoreductive therapy (13/18 responded to interferon). No deaths were observed and overall survival was similar to that of an age and sex matched control population. Our experience documents that young adults with E.T. are commonly symptomatic (54%) and the major complications occurred in 31% of cases. Ischemic complications are more frequent than hemorrhagic (6/17 vs 2/17). The factors that were
found to be predictive for ischemic events were sex (male) and cardiovascular risk factors. Moreover, in our experience, E.T. in young women is associated with an increased risk of abortion (8 abortions/15 pregnancies) especially in the first trimester (6/8).

**PO400**

**INCREASED CIRCULATING LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN ESSENTIAL THROMBOCYTHEMIA**

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An increased bone marrow (BM) vascularity has been recently described in essential thrombocytemia (ET). In order to investigate whether angiogenic cytokines may surrogate the increased BM angiogenesis we measured circulating levels of vascular endothelial growth factor (VEGF) in 47 ET patients. Sera and plasma taken at the time of diagnosis and frozen at –70°C were analyzed for the presence of VEGF using a commercial immunoenzymatic assay (ELISA)(Quantikine R&D). Results were compared with those of 60 healthy controls selected for statistical comparison. Confirming that plasma is more suitable than serum for VEGF circulating measurements especially when dealing with hematological malignancies, we found a close correlation between platelet count and serum levels of VEGF (R=0.431; p=0.002) which disappeared when dealing with plasma (R=0.129; p=0.387). On the other hand, VEGF plasma levels of ET patients were significantly higher (101.0 ng/mL; range, 3.9-502) than those of controls (24.05 ng/mL; range, 0-138; p<0.0001; Figure 1).

**Table 1.** IFN schedule for ET.

<table>
<thead>
<tr>
<th>Induction</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN: IUx10⁶/day</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>day per week/Mo</td>
<td>3/5</td>
<td>6/3</td>
<td>12/2</td>
</tr>
</tbody>
</table>

IU = international unit; Mo = months

All patients fully satisfied PSVG diagnosis criteria: 18 not previously treated; 2 treated by HU with a wash out period before starting IFN. The median observation period was 5 years (1±13).

**Table 2.** Patients characteristics.

<table>
<thead>
<tr>
<th>F/M</th>
<th>Age</th>
<th>Splenomegaly</th>
<th>Chr. abn.</th>
<th>Plts</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/11</td>
<td>38 (23±63)</td>
<td>11/20</td>
<td>1/20</td>
<td>802 (541±1458)</td>
</tr>
</tbody>
</table>

| F/M = female/male; 1 = by ultrasound scan; Chr. abn. = chromosomal abnormality found was 11q- |

**Table 3.** Results.

<table>
<thead>
<tr>
<th>Evaluable</th>
<th>Responders</th>
<th>CR</th>
<th>PR</th>
<th>Discontinued(1)</th>
<th>Off therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>18/20 (90%)</td>
<td>18/18 (100%)</td>
<td>16/18 (88%)</td>
<td>2/18 (11%)</td>
<td>2/20 (10%)</td>
<td>5/18 (27%)</td>
</tr>
</tbody>
</table>

1 = low compliance; CR and PR: complete and partial remission.

Bone marrow fibrosis was evaluated in 18 of 20 patients and was mild in 13, moderate in 4 and severe in 1. Bone marrow fibrosis re-evaluated during the follow-up in 9 patients: it was unmodified in 7 and improved in 2. We want to stress that in ET IFN: a. is a treatment that may lead to a significant percentage of off-therapy patients. Few cases off-therapy after busulphan have been reported, but this drug has leukemogenic risks; b. can maintain CR with low weekly doses, improving the compliance in a chronic disease otherwise requiring continuous therapy with cytotoxic agents; c. does not modify bone marrow fibrosis, or in some cases improves it. Because of cost and side-effects, IFN treatment of ET is still a debated argument. Our study confirms a high rate of response and the possibility of maintaining disease control with reduced doses and even after drug discontinuation. The new pegylated IFN molecules may offer additional advantages in terms of efficacy and compliance.
PO402
LONG-TERM EVALUATION OF 164 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA TREATED WITH PIPOBROMAN: OCCURRENCE OF BLASTIC TRANSFORMATION

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Introduction. Essential thrombocythemia (ET) should not be considered an indolent disease, but it may progress during its natural course into acute leukemia. Therefore, the impact of myelosuppressive agents in the blastic transformation of ET cannot be excluded. The purpose of this study was to determine the incidence of blastic crisis (BC) in patients with ET, treated with pipobroman (PB). Patients and Methods. One hundred and sixty-four patients with ET were diagnosed from April ‘76 to February ‘95. All patients received PB as first-line treatment at a dose ranging from 0.2-1 mg/kg/day according to platelet count. The patients were evaluated for the response to treatment with PB, and the occurrence of BC. Results. Mean treatment time was 164 months (range 5-244), mean initial dose of PB was 62.5 mg/day (range 25-100 mg). Dose ranging between 0.2-1 mg/kg/day according to platelet count was kept until a stable platelet count below 500 × 10^9/L was reached. Maintenance therapy was performed at a doses ranging between 0.2-1 mg/kg/day according to platelet count. The patients were evaluated for the response to treatment with PB, and the occurrence of BC. Results. Mean treatment time was 164 months (range 5-244), mean initial dose of PB was 62.5 mg/day (range 25-100 mg). Platelet count below 500 × 10^9/L was reached in 143/164 patients (87%) in a mean time of 7.5 weeks (range 3.5-10). Maintenance therapy was carried out in all respondent cases according to platelet count (mean daily dose: 25 mg, range 7-75 mg). At time of our last evaluation 152/164 patients (93%) were still alive. The overall survival (OS) and the event free survival (EFS) at 120 months were 95% and 97%, respectively. Blastic crisis was observed in 9 patients (5.5%), after a mean treatment time of 153 months (range 79-227). Hematologic features and PB doses at beginning and during maintenance therapy of the 9 patients did not significantly differ from those of the other patients. All 9 patients died after a mean time of 5.8 months (range 1-15) from the BC onset. Conclusions. The incidence of BC in our group of patients treated with PB, an alkylating agent, was comparable with the data reported in the literature. The role of myelosuppressive agents in the blastic transformation of ET could be still assessed.

PO403
LONG-TERM FOLLOW UP OF α-INTERFERON IN THE TREATMENT OF POLYCYTHEMIA VERA

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Recombinant α 2a and 2b interferon has been demonstrated effective in the treatment of chronic myeloproliferative disorders (MPDs). Between 1990 and 1994 we started a pilot study to assess the efficacy of interferon α 2a (Roferon-A) in 5 patients with polycythemia vera (PV), diagnosed according with the criteria of the Polycythemia Vera Study Group. INF therapy was initiated after normalisation of hematocrit with phlebotomies, but in one patient busulfan and pipobroman were used in addition. The median age of the patients was 46 years (range 33-59 years) and the duration of the treatment spanned from 1 to 3 years. α-INF was administered at 3 MIU/die at the beginning. Complete remission (CR) (persistent of hematocrit ≤ 45% without venesection) was obtained in few months in 1 patients while partial response (PR) was in 4 patients. Following CR or PR the INF dose was 3 MU 3 times per week. During the treatment period 4 out of 5 patients did not need venesection to control hematocrit. Only one patient was treated with flebotomy in 3 different occasions. All these patients obtained reduction of vasomotor symptoms, pruritus and splenomegaly. Early mild toxicity (flu-like syndrome) was observed during the period of treatment. The table below shows the results after the treatment with INF. The following parameter are depicted: RBC count (10^12/L), Hct (%), Hb (g/dL), WBC count (10^9/L), PLT count (10^9/L). All values are expressed as mean±SD.

<table>
<thead>
<tr>
<th>Pts.</th>
<th>RBC</th>
<th>Hb</th>
<th>Hct</th>
<th>WBC</th>
<th>PLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. 1</td>
<td>6.39±0.15</td>
<td>15.2±0.38</td>
<td>46.6±0.9</td>
<td>7.45±1.1</td>
<td>264±49</td>
</tr>
<tr>
<td>P. 2</td>
<td>4.99±0.22</td>
<td>15±0.65</td>
<td>44.6±1.9</td>
<td>5.81±1</td>
<td>139±16</td>
</tr>
<tr>
<td>P. 3</td>
<td>5.18±0.19</td>
<td>16.6±0.60</td>
<td>47.9±1.7</td>
<td>6.03±0.9</td>
<td>184±23</td>
</tr>
<tr>
<td>P. 4</td>
<td>5.72±0.24</td>
<td>16.6±0.70</td>
<td>48.6±2</td>
<td>4.36±1.75</td>
<td>191±25</td>
</tr>
<tr>
<td>P. 5</td>
<td>7.91±0.50</td>
<td>15.5±1</td>
<td>47.5±3</td>
<td>20.44±7</td>
<td>320±50</td>
</tr>
</tbody>
</table>

Conclusions. In our experience α-INF has been demonstrated effective in controlling excessive erythrocytosis and in ameliorating symptoms related to PV, but unfortunately all patients relapsed after interruption of the treatment. Advantages of INF therapy are lack of leukemogenic and teratogenic effects. Because of this, we think that in the future, trials comparing different schedules of INF therapy, including the possibility of long-term treatment regimes, with or without the potential benefit of new preparation (PEG-INF), should be taken into consideration.

PO404
COBALT AND ERYTHROCYTOSIS RELATED TO LONG STAY AT HIGH ALTITUDE

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The efficacy of cobalt as an erythropoiesis stimulator has been previously reported. Recently it has been indicated as a possible cause of chronic mountain sickness (CMS), observed in subjects living at high altitude and due to an altered adaptation to hypoxia. CMS is characterized by an over-response of physiological adaptive mechanisms to high altitude causing the appearance of a severe erythrocytosis. Therefore CMS represents a study model for disorders due to a strong stimulation of erythropoiesis. During an international scientific expedition recently accomplished in Cerro de Pasco, Peru (4,330 m.o.s.l.) focused on hematologic, cardio-pulmonary and circulatory parameters related to
the excessive erythrocytosis, we examined cobalt serum levels in 32 subjects continuously living above 4,000 m.o.s.l., 15 of whom affected with CMS and 16 with physiological reactive erythrocytosis; all subjects were males. Hematologic parameters were examined immediately after harvest with a manual technique and subsequently confirmed with an automatic counter. Serum was frozen in liquid nitrogen vapor and examined in our laboratory, in Italy (Table 1).

Table 1.

<table>
<thead>
<tr>
<th>n.</th>
<th>Median age years (range)</th>
<th>Ht%± DS</th>
<th>Cobalt µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>41 (29-51)</td>
<td>65±7</td>
</tr>
<tr>
<td>B</td>
<td>16</td>
<td>35 (22-54)</td>
<td>54±5</td>
</tr>
</tbody>
</table>

A: CMS subjects; B: high altitude subjects without CMS; SD: standard deviation.

Serum levels of cobalt did not appear different in the 2 groups and in no cases it resulted above the normal range (0.08-0.4 µg/L). In our study physiological erythrocytosis related to high altitude and pathological erythrocytosis observed in CMS patients seem not to be related to cobalt levels. It could be of interest to evaluate a possible role of the cobalt in the diagnosis of erythrocytosis due to drug misuse.

On July 1999 a 29 years old female was referred to our hospital for severe headache. The hemogram showed normal white and red cells count, while platelet count was 1,850,000/micro-liter. No vascular occlusions were shown by CNS TAC scan and M.R. No myeloid immature cells were seen in the peripheral blood smears. The bone marrow biopsy documented hypercellularity, megakaryocytic hyperplasia without fibrosis. Chronic myeloid leukemia was excluded on the basis of normal karyotype and absence of BCR/ABL gene rearrangement. The ferritin level was normal. Accordingly, the diagnosis was essential thrombocythemia. The platelet count was reduced by hydroxyurea. However the patient spontaneously stopped the treatment on February 2000 because she planned a pregnancy. She came back to our observation on May 2000, during the fourth week of pregnancy; the platelets count was 1,820,000/µL. At that time low dose aspirin (100 mg/die) was started. During the pregnancy platelet count gradually decreased until the fourth month to a plateau of about 1,000,000/µL. During the ninth month platelets count increased up to 1,520,000 in the last week of pregnancy. Aspirin was stopped and prophylaxis of deep venous thromboembolism with low weight heparin was started. She delivered at term a normal baby without hemorrhagic or thrombotic complications. The neonate had a normal hemogram. Low molecular weight heparin was stopped a week after the delivery and low dose aspirin was reintroduced (100 mg/die); afterwards therapy with hydroxyurea was restarted because of the rapid increase of platelet count and the reappearance of severe headache. In conclusion, on the basis of a limited number of observations reported in the literature, we decided not to treat our patient at the beginning of pregnancy with chemotherapeutic agents, in order to avoid their potential teratogenic effects, although the platelet count was superior to 1,500,000/µL. Of note, a spontaneous decrease of platelet count was observed during pregnancy.
PO046
SIMULTANEOUS DETECTION OF APOPTOSIS AND CD34 EXPRESSION IN NORMAL AND MYELODYSPLASTIC BONE MARROW

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Several studies have recently reported that bone marrow of patients with myelodysplastic syndromes (MDS) is characterized by an increased apoptosis, with a possible role in the pathogenesis of the peripheral cytopenia observable in these disorders. The question of whether the excessive intramedullary apoptosis in myelodysplasia predominantly involves the subset of progenitor stem cells or of more differentiated cells remains controversial. The aim of this study is to assess the degree of apoptosis in MDS bone marrow and its possible differences from normal marrow in relation to CD34 antigen expression. Patients and Methods. A double-labeling, two-color technique that combined TUNEL method to detect apoptosis and immunocytochemistry for CD34 antigen was used on fresh bone marrow slides. Nineteen MDS patients (6 RA, 2 RARS, 5 RAEB, 5 RAEB-t and 1 CMML; median age 65 yrs) and 11 healthy controls (median age 63 yrs) were studied. Statistical adjustments for the different sizes of CD34-positive and CD34-negative populations were used. Results. The apoptotic rate (AR) appeared significantly higher in CD34+ than in CD34− cell subset both in myelodysplastic (mean AR CD34+ 11.5%±6.5 vs. CD34− 3.2%± 5.0, p=0.007) and in normal bone marrow (mean AR CD34−negative 6.8%±2.3 vs. CD34+ 2.1%±5.5, p=0.02). In MDS bone marrow a higher AR than in normal controls was observed (mean AR MDS 11.5%±6.3 vs. controls 6.8%±2.3, p=0.01). When MDS and normal CD34-negative cell populations were compared, a greater AR in MDS CD34− negative cells was found (p=0.01), while no statistical difference in AR resulted from the comparison between MDS and normal CD34+ positive cell populations (p=0.18). Conclusions. Our results suggest that in myelodysplastic as well as in normal bone marrow the apoptotic phenomenon predominantly involves the maturing cells. The increase in apoptotic levels observable in myelodysplastic compared to normal bone marrow seems to be mainly due to an increase of apoptosis in the population of differentiated cells.

PO047
EPIDEMIOLOGIC ASPECTS OF MYELOFIBROSIS WITH MYELOID METAPLASIA IN SARDINIA, 1974-1993

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We have collected all cases of hematological malignancies (HM) newly diagnosed in the resident population of Sardinia in the period 1.1.1974-31.12.1993. Cases were searched for by a personally done examination of all registers of all medical departments and of all pathology institutions at that time operating in Sardinia; confirmation of diagnosis, date of diagnosis and demographic data of patients were obtained through a personally done consultation of clinical records. Epidemiologic evaluations were made according to the recommendations of dos Santos Silva. A total of 7339 cases of H.M. was found out. Among them, 108 patients had a diagnosis of idiopathic myelofibrosis (MMM), that we considered sufficiently supported by histological and/or cytological and/or clinical data. They represents 1.47% of all cases of hematological malignancies. Male-to-Female ratio was 1.45:1. Mean age at diagnosis was 66 years (range 29-90, median 66). Age- and sex-specific incidence rates confirmed increased incidence in advanced ages and in males. Crude annual incidence rate was highly variable (range 1-9, mean 5.4, median 5) with more cases diagnosed in the second ten years of our survey (1974-1983: range 1-7, mean 4, median 4; 1984-1993: range 4-10, mean 6.8, median 6). We think that the increase of cases of MMM observed in the second ten years of our survey, only in part explained by ageing of Sardinian population, is largely due to an increase of diagnosis of previously undetected patients, due to increased physician awareness of the disease and to an extended use of bone marrow biopsy. Age standardized rate (world population) was 0.19 × 100.000 × year for females and 0.30 for males. Such rates are lower than those reported for other populations. It is possible that many cases of MMM still remain undetected.

Acknowledgements. This research, which went on for more than twenty years, has been possible only by contributions of many people. Contributions have been of different types, different quality and different duration: but for all of them I am equally grateful. Being impossible to mention all contributors, to remember all of them, I have quoted as authors just the contribut ors of the first times and those of the last times. (GB)
EXPRESS CD 95 ANTIGEN ON THE SURFACE OF BONE MARROW CD34+ CELLS IN MYELODYSPLASTIC SYNDROMES

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*Department of Hematology; *Clinical Laboratory, “SS. Antonio e Biagio e C. Aringo” Hospital, Alessandria

Background. In myelodysplastic syndromes (MDS), ineffective hemopoiesis is supposed to result from an excessive cellular death rate charged to hemopoietic progenitors, thus avoiding their differentiation and entering the bloodstream. An increased and early apoptosis would delete the effect of the high proliferative activity and would justify the common pattern of peripheral pancitopenia. Aim of the work. To evaluate the expression of the cell-surface Fas receptor (CD95) in different marrow populations of MDS patients, in consideration of its fundamental role in the activation of the apoptotic pathway. Patients and Methods. The bone marrow samples from 30 consecutive patients affected by MDS (including all FAB subtypes: RA, RAEB, RAEB-t), were analysed for the expression of CD95 in comparison to the marrows from 5 normal controls. Flow cytometric analysis was executed on all bone marrow mononuclear cells (BM MNC), immediately after separation on density gradient and after 18 hours (t18) of incubation in RPMI 1640 added with 1% glutamine, 1% PES and 10% FCS. The CD95 expression was analysed both on all BM MNC and on the CD34+ cells. Results. Flow cytometric analysis, immediately after separation on density gradient, first allowed us to point out that MDS are characterized by a constitutive increased expression of CD95 in both all BM MNC (25% in controls vs. 44% in MDS; p value < 0.01) and in CD34+ population (1% in controls vs. 5% in MDS; p value = 0.01). Moreover the CD95 evaluation, performed after 18 hours (t18) of incubation in RPMI 1640, allowed us to point out that normal marrows are characterized by a slight not significant spontaneous increase in CD95 expression in both all BM MNC (t0 25% vs t18 28%; p value =n.s.) and CD34+ population (t0 1% vs t18 2%; p value =n.s.). On the contrary, in MDS patients, even if the difference of CD95 expression in BM MNC was not evident from t0 to t18 (t0 44% vs t18 45%; p=n.s.), a statistically significant increase was shown when the analysis was limited to the CD34+ population (t0 5% vs t18 7%; p=0.01). Conclusions. Our data lead to suppose that the marked proneness to enter the apoptotic pathway, shown by MDS cells and particularly by the CD34+ population, may be due to the constitutively higher CD95 expression in MDS compared to normal marrows. Since CD95 expression spontaneously increases only in MDS CD34+ population, further studies should be useful, based on treatment of MDS and normal mononuclear cells with cytokines as IFN-γ and TNF-α, whose serological levels are spontaneously increased in MDS patients, intended to a better comprehension of the exact role of CD95 in the CD34+ apoptotic pathway and its modulation.

MYELODYSPLASTIC SYNDROMES IN PIEDMONT. PRELIMINARY EXPERIENCE OF THE PIEDMONT MYELODYSPLASIA REGISTRY


On behalf of the Piemonte MDS Registry, Alessandria

Background. It is usual opinion that the myelodysplastic syndromes (MDS) are under-diagnosed and their incidence is underestimated by cancer registers, as a consequence of the following factors: a) MDS are a disease of advanced age and many elderly patients are not well studied; b) the diagnosis, when suspected, is often considered a secondary event in patients followed for other major non hematological age-related diseases; c) only a minority of these patients are followed by hematological institutions. Patients and Methods. In 1999 we started in Piedmont a program about MDS with the cooperation of both hematological and internal medicine departments. The major aims of the program are: 1) to define homogeneous diagnostic guidelines; 2) to collect epidemiological information; 3) to cryopreserve bone marrow cells of at least a group of patients for molecular biology studies; 4) to analyse the clinical outcome of patients homogeneously treated. The forms with historical, clinical and laboratory information have been registered from the registry data center. Results. From June 1999 to April 2001, 179 MDS patients were registered from 26 different institutions: 101 (56%) from hematology and 78 (44%) from internal medicine departments. The patients are distributed, according to the time from diagnosis as follows: 44 in 1999, 109 in 2000 and 26 in the first three months of 2001. Mean age is 73 (range 30–95), with the following distribution for class of age: ≤ 60 years 19 patients (11%); 61–70 years 50 patients (28%); 71–80 years 70 patients (39%); ≥ 81 years 40 patients (22%). One hundred and five patients (59%) are males. An important age-related comorbidity requiring drug therapy is present in 72 cases (40%). A previous cancer is reported in 23 patients (13%), a former chemotherapy in 8 patients (5%) and a radiotherapy in 12 patients (8%). Cytogenetic data are present in 122 patients (68%). The distribution of the final diagnosis according to the WHO diagnostic groups is: RA 67 (40%), RARS 10 (6%), RAEB 56 (33%), S–syn- drome 4 (2%), unclassifiable 14 (8%), CM ML 18 (11%). 122 patients are so far valuable for the IPPS prognostic score: 34 patients (28%) score 0; 66 patients (54%) score 0.5–1; 12 patients (10%) score 1.5–2; 10 patients (8%) score ≥2.5. Information about the exposure to the most common risk factors (cytotoxic drugs, radiotherapy, benzene and solvents, pesticides, ionising radiation, smoke, hair dye) are so far recorded for 141 patients (79%) for future correlations with chromosome abnormalities. Conclusions. The collection of historical and clinical information of MDS patients for future epidemiological case-control studies is possible on the basis of a spontaneous cooperation. The very advanced age of MDS patients is confirmed. A high proportion of these patients is not followed by hematology departments. The percentage of patients studied for chromosome abnormalities could be further increased.
LONG-TERM EFFICACY OF THALIDOMIDE THERAPY FOR ANEMIA OF TRANSFUSION-DEPENDENT PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Thalidomide has been recently employed for the treatment of some hematological malignancies. The exact mechanism of action of this drug has not been completely elucidated, but immunomodulating, anti-angiogenesis, anti-inflammatory or anti-apoptosis activities have been postulated. Since at least some of these mechanisms may be involved in the pathogenesis of myelodysplastic syndromes (MDS), we are currently evaluating the potential therapeutic role of thalidomide in these diseases. As of March, 2001, thalidomide has been administered to 12 patients with low or intermediate-low risk MDS (according to IPSS). Seven patients were males and 5 females; mean age was 65 yrs (range 48-81). Seven patients had refractory anemia (RA), 3 had RA with ring sideroblasts (RARS) and 2 had RA with excess of blasts (RAEB). Previous treatments mainly included steroids, folates and other vitamins, androgens, recombinant erythropoietin (alone or in combination with G-CSF, GM-CSF or amifostine). All patients were heavily transfusion-dependent (Hb < 8 g/dL), requiring 4-8 units of packed red-cell transfusions every month. Thalidomide was given on a compassionate basis, after an informed consent was achieved, at the dose of 100 mg/d per os, at bedtime, for 1 week and then progressively increased up to a maximum tolerated dose of 300 mg/d. Iron-chelating therapy was the only concomitant associated treatment. Two patients are too early for response evaluation. Four patients (three more than 75 year-old) stopped thalidomide within a few days because of relevant side effects (somnolence, constipation, general malaise, renal failure). Three further patients did not show any modification of hematological parameters and stopped the treatment after 2 months. One of them (affected by RAEB) developed secondary acute myeloid leukemia two months later, while receiving a combination of oral Ara-c and GM-CSF. The remaining 3 patients (2 RA and 1 RARS) became completely transfusion-independent within 4-9 weeks and reached stable values of Hb comprised between 9.4 and 11.4 g/dL. Due to a slight worsening of peripheral white blood cell and platelet counts, thalidomide was stopped in one of responders after 3 months of therapy. Since hemoglobin value rapidly decreased to less than 8 g/dL, thalidomide was re-started and the patient returned to be transfusion-independent. These erythroid responses are still maintained in 2 patients, after 9 to 12 months of thalidomide therapy, with adjusted doses of 150-250 mg/d, allowing them to carry out their normal occupational activities. The third responder returned to receive transfusions after 5 months of treatment, but his transfusional requirement after 8 months under thalidomide is currently reduced to about one unit every 6 weeks. Responders were all treated within 1 year from diagnosis, were less than 65-year old and showed a normal karyotype. Morphological examination of bone marrow, lymphocyte subpopulations, HbF and serum ferritin levels remained substantially unchanged throughout the study, while serum levels of endogenous erythropoietin significantly decreased, paralleling the rise of Hb. Reticulocyte count moderately increased only in one responder, thus suggesting that thalidomide could directly or indirectly reduce the apoptotic process of erythroid precursors, rather than stimulate residual normal or dysplastic erythropoiesis. In this setting, evaluation of marrow angiogenesis, serum levels of soluble transferrin receptor and angiogenic circulating growth factors are in progress. Our results indicate that thalidomide, though poorly tolerated in elderly subjects, may have relevant effects on anemia of transfusion-dependent MDS, particularly in low-risk, younger patients with a recent diagnosis.

THE INTENSITY OF EXPRESSION OF CD44 ON MYELODYSPLASTIC CD34+ CELLS CORRELATES WITH IPSS SCORE

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CD44 and its isoforms are widely expressed, multifunctional, cell-surface glycoproteins that have been implicated in the regulation of normal hematopoiesis. Furthermore, abnormalities of CD44 expression and function have also been described in leukemic patients (pts). The aim of our study was to analyze, in a three-color flow cytometric assay, the expression of CD44 on CD34+ progenitors from BM of 43 pts affected by MDS (19 RA, 1 RARS, 17 RAEB, 6 RAEBt). Due to the high co-expression of CD44 (median 98%, range 39-100), we corrected this value by the mean fluorescence intensity (MFI) of each sample, generating an adhesion molecule index (AMI = product of MFI and percent positive cells). In the whole MDS group, CD44 had a median value of 1926 (range 122-6394); the higher the bone marrow (BM) blast infiltration, the higher the CD44 AMI value (Spearman’s r=.36, p=.02). A cut-off value of 10% BM blasts distinguished 2 groups with a high difference of AMI expression (mean 1650 vs. 2644, p=.023). Furthermore, pts with ≥ 2 cytopenias had a higher CD44 AMI (mean 1396 vs. 2449, p=.022), while no difference of expression could be observed among different cytogenetic groups. Such correlations leaded to a significant difference of CD44 mean AMI among different IPSS score risk groups (low-risk 1320 vs. Int-1 1610 vs. Int-2 2957 vs. High-risk 3526, p=.014). In conclusion, the intensity of CD44 expression identifies high-risk IPSS pts. The future challenge is to determine whether this molecule may contribute to the perturbation of hematopoiesis observed in MDS.

RECOMBINANT ERYTHROPOIETIN AS A TREATMENT FOR "LOW RISK" MYELODYSPLASTIC SYNDROMES: RESULTS OF A STUDY

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Background and Aims. Since the end of 1999 the Italian Ministry of Health allowed the use of recombinant erythropoietin (rEpo) as a treatment of low risk myelodysplastic syndromes (MDS). The aim of the present study was to evaluate the clinical
usefulness of this treatment in an open phase 3 single-center study. Patients and Methods. Since January 2000 to December 2000 43 patients (23 males; 20 females; median age 72 y) suffering from low risk MDS (31 RA; 9 RARS; 3 RAEB with blasts < 10%) and with hemoglobin levels (Hb) < 10 g/dL (median 6.05) were enrolled into the study. Fifteen of 43 (35%) were transfusion-dependent at the beginning of the study. RHEpo was administered s.c. at the dose of 10,000 IU 3 times a week for at least 3 months before efficacy evaluation, and was stopped in the case of no response. In responders patients, RHEpo was continued until response was maintained. Throughout the study side effects possibly due to RHEpo therapy were recorded. Results and Conclusions. Thirty-eight out of the 43 patients initially enrolled into the study were evaluable for hematological response after 3 months of treatment with RHEpo: on an intention to treat basis, 21/43 patients (48.8%) responded favorably to RHEpo treatment. According to FAB classification, a positive clinical (either major or minor) response was observed in 18/31 (58%) patients with RA and in 3/9 (33.3%) patients with RARS, while none of the 3 patients with RAEB responded to RHEpo. Among the 21 responders to RHEpo, 19 (90%) still positively responded 6 months after the beginning of RHEpo treatment; 9 and 6 of them, at the time of present analysis, were still treated with RHEpo and in continuum response after 9 and 12 months, respectively. According to transfusion requirement, 55% of not transfusion-dependent patients responded to RHEpo treatment, whereas only 40% of the transfusion-dependent subjects did so. No adverse effects were recorded in any of the patients enrolled into the study, and the only reason for the stopping of RHEpo treatment was the lack of response. In conclusions, present data confirm the efficacy of RHEpo as a treatment for anemia associated to low risk MDS. Its administration is well tolerated and free from side effects. Because of the high cost of the drug and the lack of a more definite evaluation of other clinical indicators of clinical response (such as, physical examination, quality of life) the cost/benefit ratio of RHEpo remains to be determined in the setting of MDS.

A RANDOMIZED STUDY OF α-INTERFERON VS. α-INTERFERON AND LOW DOSE ARABINOSYL CYTOSINE IN CHRONIC MYELOID LEUKEMIA

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The introduction of α-interferon (α-FN) has significantly improved the survival in chronic myeloid leukemia (CML) but some patients do not respond and many responses are not durable. To improve the results, α-FN has been associated with other treatments but so far only the association with low dose arabinosyl cytosine (LDAC) has been shown to increase the response rate and to prolong survival. This has been found in a multicenter French study. We report here the results of a study similar to the French one, in which 338 patients with early chronic phase Ph+CML were assigned at random to treatment with α-FN alone or in combination with LDAC. The scheduled dose of α-FN was 5 MIU/m2/day and the average administered dose ranged between 0.82 and 0.66 of scheduled. The scheduled dose of LDAC was 40 mg/day for 10 days, monthly, and the average administered dose decreased from 0.82 to 0.26 during the study. The primary efficacy endpoints were complete hematologic response rate at 6 months (62% in the α-FN+LDAC arm vs. 55% in the α-FN arm, p=0.11), major cytogenetic response (MCR) rate at 24 months (28% vs. 18%, p=0.003) and overall survival (5-year survival 68% vs. 65%, p=0.77). Overall survival was not affected by treatment also within different prognostic risk groups (Sokal low, intermediate or high). Also the duration of MCR was identical. The results of this study confirm the results of the French study only for the response rate but not for the survival, suggesting that the relationship between the cytogenetic response and survival may be very variable and that a meta-analysis of these and other studies of α-FN vs. α-FN+LDAC is required to settle the issue of the role of LDAC in the treatment of CML.

today in molecular remission. Before MYLOTARG he received idarubicin 110 mg/m², cytarabine 18,500 mg/m², mitoxantrone 122 mg/m², etoposide 500 mg/m² and 12 Gy TBI, requiring transfusional support with 40 erythrocytes and 58 platelet units. MYLOTARG and filgrastim courses induced aplasias lasting 18 and 11 days; a support of 5 platelets units and an increase of AST levels, which reverted to normal within few days. Our case report suggests the feasibility of a successful therapy, with short hospital admission and mild untoward effects, despite previous heavy and repeated treatments in patients with additive non-hematological complication.

P0415

SERUM CHOLINESTERASE IS AN EARLY AND SENSITIVE MARKER OF GRAFT VERSUS HOST DISEASE


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Serum cholinesterase (CHE) has been reported to be a significant indicator of liver function and of prognosis in patients with cirrhosis. On the other hand liver complications are frequent following allogeneic stem cell transplantation (HSCT). We therefore tested whether CHE was predictive of graft versus host disease and outcome in HSCT recipients. We studied 882 patients receiving a HSCT from an HLA identical sibling (SIB) (n=691), an alternative donor (n=191) or a syngeneic twin (n=6). Acute graft versus host disease (GVHD) was scored as grade 0-I, II, III-IV in 427 (48%), 352 (40%), and 103 patients (12%) respectively. Overall, 239 patients died of transplant related complications (TRM). On day +7 the median CHE serum level was 5.9×10³ IU/L in patients who survived or died of TRM. On day 0 serum CHE levels were 2.3 and 2.1×10³ (p=ns) indicating the impact of the conditioning regimen. On day +7 after HSCT the median level for surviving patients was 2.6×10³ IU/L vs 2.3×10³ IU/L for patients who subsequently died (p=0.0002). The difference became greater with time: on day +21 CHE levels were 3.3 vs 2.5×10³ IU/L (p<0.00001) on day +100 4.2 vs 2.3×10³ IU/L (p=0.00001). On day +21 CHE levels were 3.3 vs 3.0 vs 1.9×10³ IU/L for patients with GVHD grade 0-I, II, and III-IV respectively (p<0.0001). Serum CHE levels were highest in twin grafts, significantly more than SIB grafts with no GVHD. SIB grafts with GVHD grade II had similar CHE levels as alternative graft with GVHD grade 0-I. Patients who would subsequently relapse had significantly higher CHE levels on days +50 (p=0.0003) and +100 (p=0.0001), but not thereafter. In conclusion serum cholinesterase is a simple and reliable marker of acute GVHD and transplant related complications: it is also an indicator of alloreactivity and leukemia relapse.

P0416

HEMOPOIETIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKEMIA. THE EFFECT OF DONOR TYPE AND CONDITIONING INTENSITY


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We report the outcome of allogeneic HSCT in 200 patients with CML grafted from an HLA identical sibling (SIB, n=97) or an alternative donor (ALT, n=103). Median follow up for patients surviving the transplant are 1311 days for SIBs and 1283 for ALT grafts. The actuarial 10 year survival was 63% for SIB and 56% for ALT donor grafts (p=0.03), the transplant related mortality (TRM) was 36% vs 44% (p=0.03) and the relapse rate 40% vs 30% (p=0.4). For patients in chronic phase the figures for SIBs vs ALT grafts are: survival 68% vs 59% (p=0.1), TRM 27% vs 35% (p=0.1), relapse 33% vs 8% (p=0.008). We used 3 different intensity regimens: conventional (CY-TBI, n=126), conventional (CY-thiotepa, n=43) and reduced (CY-thiotepa, n=31). In multivariate analysis survival and TRM an alternative donors is a unfavorable factor (p<0.03), whereas on relapse the low intensity regimen has the major unfavorable impact (p<0.0001). This study suggests that recipients of an alternative donor transplant have a borderline higher risk of TRM following an allogeneic HSCT. It also shows that the intensity of the conditioning regimen has an important impact on control of the underlying disease.

PO417

DONOR LYMPHOCYTE INFUSIONS (DLI) FOR PATIENTS RELAPSING AFTER HEMOPOIETIC STEM CELL TRANSPLANTATION: ANALYSIS OF FACTORS PREDICTING GRAFT-VERSUS-HOST DISEASE AND RESPONSE IN 593 DLI IN A SINGLE CENTER


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In the present study we analyse factors predicting GVHD and response after donor lymphocyte infusions (DLI). One hundred patients received 593 DLI between June 1990 and December 2000 in a bulk dose (n=13) or in escalating dose infusions (n=87). Fifty one patients had chronic myeloid leukemia (CML) and 49 an other hematologic malignancy. The actuarial probability of achieving a remission at 3 years was 100% for CML patients with cytogenetic relapse, 78% for CML in CP, 54% for CML accelerated phase, 100% for acute leukemias/MDS with minimal residual disease (cytogenetic, molecular relapse) and 0% for other hematologic malignancies (p=0.001). Actuarial 10 year disease free survival was 43% for CML and 14% for other malignancies. Acute graft versus host disease (aGVHD) (grade II – IV) occurred in 21 patients (21%). Factors predicting response in multivariate analysis were diagnosis and number of CD3+ cells infused. Serum γ glutamyl transferase (γGT) on day +21 after DLI was increased in patients who developed GVHD (p<0.0001) and proved a significant predictor of GVHD in multivariate analysis. The actuarial probability of treatment related mortality was 9% for HLA identical siblings and 44% for alternative donor transplants (p=0.006). DLI can induce durable remissions in CML patients and some acute leukemias with minimal residual disease. DLI may be used as consolidation of remission achieved after induction chemotherapy. The efficacy of DLI in lymphoproliferative disorders is less clear. GVHD correlates with the number of CD3+ cells infused and can be predicted by serum γGT levels.
TEN YEAR FOLLOW UP OF A RANDOMIZED STUDY COMPARING CYCLOSPORIN 1 mg/kg vs 5 mg/kg AS GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN ALLOGENIC BONE MARROW TRANSPLANTATION

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We have shown some years ago that the intensity of GvHD prophylaxis, following allogeneic BMT, has a significant impact on leukemia relapse (Blood 77; 1423-1428, 1991). In that study 81 patients with acute leukemia (AML=44; ALL=37; 1stCR=53; >1st CR=28) were randomized to receive cyclosporin (CyA) 1mg/kg i.v. or 5 mg/kg from day -1 to day+20. It is important to note that the average CyA serum levels were significantly different in the two arms only from day-1 to day +10 (295 vs 686 ng/mL; p=0.004), but not between day +11 and +20 (465 vs 650 ng/mL, p=0.1); this is due to the fact that patients in the CyA 1 mg arm had their dose increased beyond day +10 due to acute GvHD and patients in the CyA5 mg arm had their dose decreased due to toxicity. Therefore administered dose and serum levels were both significantly different only for 10 days. The median follow up for surviving patients is now 11.7 years (10.2-13 years). The actual 10 year transplant related mortality (TRM) in the CyA 1 mg vs 5 mg is respectively 36% vs 28% (p=0.4). The relapse rate is 17% vs 43% (p=0.008) and the survival is 48% vs 28% (p=0.07). For patients in 1stCR the relapse rate in the two arms was 11% vs 36% (p=0.02) and for advanced disease it was 28% vs 57% (p=0.1). In multivariate analysis for TRM the only significant predictor was male recipient, for relapse the predictors were phase of the disease and randomisation arm, and for survival it was phase of the disease. This study confirms that a difference in the intensity of the CyA dose in the first 10 days following an allogeneic bone marrow transplant in acute leukemia, has significant implications for the maintenance of a durable remission 10 years following BMT.

THE EFFECT OF ANTITHYMOCYTE GLOBULIN FOR GRAFT-VERSUS-HOST DISEASE PROPHYLAXIS IN 119 ALTERNATIVE DONOR TRANSPLANTS

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We have analysed the outcome of 119 patients undergoing an alternative donor transplant: 61 patients received no ATG in the conditioning regimen and 56 received ATG in a dose ranging from 7.5 to 15 mg/kg in the conditioning regimen. 13 were family donors partially mismatched and 106 were unrelated donors. The conditioning regimen was either CY-TBI for all patients. The incidence of acute grade III-IV GvHD was 43% vs 15% for patients receiving no ATG vs patients receiving ATG (p=0.001). The incidence of extensive chronic GvHD was 63% vs 37% in the same two groups (p=0.02). Transplant mortality (TRM) was 46% vs 38% (p=0.3), relapse 11% vs 14% (p=0.7) and survival 49% vs 50% (p=0.8). We conclude that the use of ATG in the conditioning regimen significantly reduces the incidence of acute and chronic GvHD. TRM is however only modestly reduced, and survival unchanged. The quality of life of patients without cGvHD may however be superior. Alternative approaches to the problem of GvHD prophylaxis, such as pre-emptive theropy should be explored.
PUBLISHED ABSTRACTS

PUB001
IMMUNOPHENOTYPE ANALYSIS IN 119 PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) FOLLOWING A PREVIOUS MALIGNANCY: A COMPARISON WITH IMMUNOPHENOTYPE OF 231 DE NOVO AML


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In a multicenter GIMEMA study, the immunophenotype pattern of acute myeloid leukemia following a previous malignancy (PM) in comparison with that of de novo AML was evaluated in order to identify possible differences. Between June 1992 and December 2000, 350 cases of adult AML were recorded. Data regarding immunophenotype of 119 AML following PM were matched with those of 250 patients with de novo AML (ratio 1:2) with similar age and morphological characteristics. The PM most frequently represented were breast cancer, followed by Hodgkin's disease and lymphoma. In 37 patients surgical resection was the sole therapy for PM, while the other patients received radio and/or chemotherapy. Comparing AML following a PM with de novo AML a significant difference in the percentage of cases expressing the following antigens was found: CD4 (p<0.002), CD16 (p<0.02), CD22 (p<0.02), CD33 (p<0.006), CD117 (p<0.04), HLA-DR (p<0.01). No differences were found for the other monoclonal antibodies. Comparing the immunophenotype distribution in the patients with AML following a PM treated with surgery alone with those of patients treated with chemo-radiotherapy no differences were found. None of the antigens tested were associated with treatment outcome. Immunophenotype analysis in this large series of AML following a PM showed differences in antigenic expression compared to de novo AML. The absence of differences between AM following a PM treated with surgery vs chemo and/or radiotherapy and the reported differences in immunophenotypic profiles suggests that AML following a PM and de novo AML could be distinct disease entities; immunophenotypes could be useful to distinguish de novo from AML following a PM. The biological and the clinical significance of these data, however, need further evaluations.

PUB002
ACUTE PROMYELOCYTIC LEUKEMIA (VARIANT FORM) WITH MARROW FIBROSIS

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PUB003
MAINTENANCE TREATMENT WITH LOW DOSES OF ARA-C IN ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS

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Acute promyelocytic leukemia (APL) is characterised by the presence of atypical promyelocytes, filled of Auer rods. The specific chromosomal translocation t(15;17) determines the fusion gene PML/RAR. The variant form of APL (M3v) has hypogranular blasts, devoid of Auer Rods; nuclei are polymorphic and often bilobed. Both types of APL share an identical clinical picture and are dramatically responsive to All Trans Retinoic Acid (ATRA). The authors describe two cases of M3v that have as distinctive characteristic the presence of marrow fibrosis. Case #1: F, 67 years. The patient had a history of breast cancer, developed 18 months before, and was treated by quadrantectomy and radiotherapy. At admission, she was pale and she presented faint bruising. Laboratory tests revealed peripheral pancytopenia (WBC 0.8×10^9/L, PMN 0.2×10^9/L, platelets 21×10^9/L, Hb 4.5 g/dL) and no coagulative abnormalities. On examination of peripheral blood, rare blasts with M3v morphology were observed. The marrow biopsy showed an hypercellular marrow, with areas of reticulin fibrosis, replaced by blasts that, at the imprint of the biopsic specimen, presented the typical morphology. An immunohistochemical analysis, performed on the marrow biopsy with the MoAb PG-M3, was positive. Immunophenotyping of a peripheral blood specimen was typical of the M3v and the diagnosis was confirmed by detection of the transcript of PML/RARα. The patient was treated according to the AIDA protocol and after six weeks achieved the morphological and molecular complete remission that is maintained to date, 6 years later. Case #2: F, 45 years. The patient presented with fatigue and ecchymoses lasting for two months. At admission, the hemogram showed pancytopenia (WBC 1190×10^9/L, platelets 8×10^9/L, Hb 4.5 g/dL). The examination of peripheral blood smears showed blasts with M3v morphology. No coagulative abnormalities were detected. The bone marrow was aspirable. The bone marrow biopsy showed the massive presence of blasts and diffuse fibrosis. Immunocytochemistry performed on peripheral blood smears with the MoAb PGM 3 was positive. The transcript of PMR-RARα was demonstrated on peripheral blood. On the immunophenotypic analysis, the blasts were positive for CD33, CD13 and MPO and negative for CD34 and HLA-DR. The patient was treated with ATRA and idarubicin, according to the protocol AIDA 2000, and achieved complete remission after 30 days. The two cases share some clinical and biological aspects that are unusual in FAB M3v, as the leukopenia and the marrow fibrosis. The last topic is exceptionally rare in APL and was described to date only once from Japanese authors who hypothesized the causative effect of TGF-β in inducing fibrosis. At our knowledge there is no reports about cases of M3v associated to marrow fibrosis. This pattern is not detrimental because both patients obtained the complete remission, one long-lasting.
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Although risk of systemic fungal infection following treatment for acute leukemia is high, isolated splenic candidiasis is unusual and only few cases are reported in the literature. We discuss a patient with acute lymphoblastic leukemia (ALL) who developed a splenic candidiasis which was successfully treated by a laparoscopic splenectomy. A 41 year-old-female was referred to the Section of Hematology on September 2000 for lymphadenopathy in the lateral region of the neck and leukocyte count of 21×10^9/L with 60% blast cells. Bone marrow aspirate revealed replacement of normal elements by 80% lymphoblasts and massive melena. A second ALL patients with typical FAB L3 morphology is reported. First case presented with Acute Lymphocytic Leukaemia (ALL) of early B-cell phenotype showing FAB-L3 morphology are reported. First case was observed in September 1997 and concerned a female, 69 years old, who was admitted because of generalised malaise and pancytopenia. The evaluation of bone marrow aspirates showed the massive presence of blasts showing prominent aggregates of nuclear chromatin, one or more nucleoli, and a deeply basophilic cytoplasm containing numerous vacuoles of varying sizes and shapes. Immunophenotypic analysis revealed that the blasts were positive for TdT, CD34, CD19, CD20, CD22 markers and negative for CD10 and surface immunoglobulins (SIg). Chromosomal and molecular phenotype showed no abnormalities. Lumbar puncture provided no presence of blasts in cerebrospinal fluid. Diagnosis of pre B-cell lymphoid leukaemia was made and patient was treated according to the 0183 GIMEMA ALL trial protocol. The patient achieved a complete remission after induction therapy, containing Vincristine, Prednisone, 6 Mercaptopurine and Methotrexate, disease relapsed with a massive involvement of bone marrow, peripheral blood and central nervous system (CNS). An aggressive salvage regimen was promptly started but resulted ineffective. Patient died two months later because of diffuse aspergillosis and massive menela. A second ALL patients with typical FAB L3 morphological features, a man of 76 years old, is followed in our Centre from February 2001. The immunophenotyping of leukemic blasts showed positivity of CD19, CD34 markers and TDT activity in the absence of SIg and others lymphoid antigens. No cytogenetic abnormalities were found. The clinical presentation was characterised by pancytopenia in absence of extramedullary and CNS involvement. Patient presented with a poor performance status and was not eligible for aggressive regimen. The induction therapy consisted only of weekly administration of Vincristine (2 mg) and daily Methylprednisolone (100 mg) for six weeks. Patient achieved a partial response and actually is managed with palliative treatment containing daily oral 6-Mecaptopurine and week-
Microcytosis, defined as mean corpuscular volume (MCV) <82 fL, is a highly prevalent finding during blood examination (nearly 3% of patients admitted in hospital). In the majority of cases, it is the result of impaired hemoglobin synthesis (disorders of iron metabolism and porphyrine and heme synthesis, as well as impaired globin synthesis). The investigation of microcytosis represents a medical challenge: the cause often remains unexplained, the most frequent concomitant pathologies (iron deficiency, anemia of chronic disorders, thalassemia trait) have different diagnosis, prognosis and therapy, and there are also mixed situations. Aim of this study was to evaluate microcytosis incidence and diagnostic process by pathologist. We evaluated 4497 complete blood cell count (1117 unselected hospitalised patients and 3380 unselected outpatients) admitted to our laboratory during two consecutive weeks by ADVIA™ 120 Bayer hematological analyser: 227 (5.0%) samples with microcytosis, 62 (5.5%) hospitalised patients and 165 (4.8%) outpatients, 74 (32%) male and 153 (67%) female, aged 0.5 to 95. 144 patient samples were eligible for the study and subdivided into five pathology groups: iron deficiency (IDA), thalassemia trait (TT), anemia of chronic disorders (ACD), IDA+ACD (COMBI) and OTHER on the basis of clinical and laboratory criteria (HbA2, HbF, ETFHb, serum ferritin, α1 glycoprotein, CRP, TRF index). Using the routine red blood cell counts and indices [RBC, Hb, MCV, MCH, MCHC, RDW, HDW, percentage of hypocromic RBCs (%ipo), percentage of microcytes (%MC), microcytosis ratio (MC/MCRatio)] and reticulocyte count (reticulocytes, % reticulocytes, IRF M+H, MCVr, CHCMr, CHMr) statistical significant differences (p<0.0001) were observed only between the IDA and TT groups for RBC count (1248±104 µL), MCV (10.55 fL), MCH (3.56 pg), %MC (19.48%), MCR (10.69 fL), CHr (3.17 pg) and between IDA and ACD for %ipo (21.31%) and MCR/MCP ratio (9.81). Besides a good diagnostic performance estimated by ROC curves was found only to distinguish between IDA and TT: the best discriminating parameter (AUCROC 0.941) was found to be MCR/MCP ratio with a cut-off of 1.08 (90.4% sensitivity, 88.3% specificity) and then MCV (AUCROC 0.936, 69.1 cut-off, 90.4% sensitivity, 88.3% specificity, 90.4% specificity, 88.9% specificity). RBCC (AUCROC 0.931, 5.20 cut-off, 90.4% specificity, 89.4% specificity, 94.4% specificity), %MC (AUCROC 0.921, 91.3 cut-off, 83.7% sensitivity, 94.4% specificity, 94.4% specificity) and MCV (AUCROC 0.900, 17.8 cut-off, 84.6% sensitivity, 72.2% specificity) while less effective were MCH and CHr; for the distinction between the IDA and ACD or COMBI groups and between the TT and ACD or COMBI groups no effective parameters were found. This study shows at first sight that some simple hematological parameters allow to suspect, in the presence of microcytosis, thalassemia trait rather than an iron deficiency and addressing in this way the diagnostic and therapeutic management.

Concomitant laparoscopic cholecystectomy and splenectomy for surgical management of hereditary spherocytosis

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Hereditary spherocytosis is the most common red blood cell membrane disorder and often is associated with haemolytic crisis and premature cholelithiasis. There is commonly a family history and a typical clinical and laboratory picture so that the diagnosis is usually made without additional laboratory tests. Splenectomy is the only effective therapy for this disorder and
often it is performed in combination with cholecystectomy. Minimally invasive surgery has recently gained acceptance as the surgical approach of choice for a variety of surgical disorders in children and young. Although traditional open surgery is still regarded as the standard approach for a splenectomy in young when necessary for hematological disorders a few cases of successful laparoscopic splenectomy (LS) have been reported. Conventional surgery requires a wide upper abdominal incision for correct exposure of the gallbladder and spleen. Laparoscopic cholecystectomy and splenectomy have been performed safely worldwide. We report our experience with our patient 20 ys old who underwent combined laparoscopic splenectomy and cholecystectomy because of hereditary spherocytosis with persistent anemia, cholelithiasis and splenomegally. The cholecystectomy was performed in a standard laparoscopic fashion and was performed first, then splenectomy was achieved and the spleen removed by morcellation into a retrieval bag. The patient not required conversion to open technique or blood transfusion, the diameter of the spleen was less than 15 cm and the operative time was 200 minutes, moreover the blood loss was less than 150 mL and postoperative hospital stay 4 days. No major complications occurred in our case, cosmetic results were judged excellent and the patient showed sharp hematological improvement until 24 months follow-up. We conclude that combined laparoscopic approach is safe and effective for treatment of hereditary spherocytosis, and we think that appropriate preoperative preparation of patient, meticulous surgical technique and careful postoperative care were key factors in obtaining the same long-term results as with open surgery.

PUB009
ADJUSTED BLOOD SEPARATOR PROGRAMS TO HARVEST AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS IN UNSELECTED POOR PROGNOSIS HEMATOLOGIC MALIGNANCIES

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High dose therapy with peripheral blood stem cell (PBSC) rescue may represent a chance in poor prognosis patients affected by hematologic malignancies, high risk at onset or requiring more lines of therapy to obtain a response. This approach might be limited by reduced bone marrow reserve, due to heavy previous treatments, making inadequate stem cells mobilization and collection. Nevertheless, the opportunity offered by tailored stem cell collection programs can overcome the impediment. Therefore we have evaluated the PBSC collections performed with continuous flow blood separator Fresenius AS 104, applying the MNC PLY or C4Y PBSC LYMPh programs, in 21 unselected poor prognosis patients: NHL 6 (CR 2,PR 4), HL 4 (PR), MM 7 (CR 3,PR 4), ANLL 2 (CR), ALL Ph 1+2 (CR); median age 50 yrs (range 20-65). Stem cells were mobilized by cyclophosphamide 4 g/m2 (6 pt) or cyclophosamide 7 g/m2 (7 pt) or antracycline containing schedules (6 pt) plus G-CSF 5 microg/kg/die. We have collected median dose of 11.4×10^6/kg CD 34 cells (range 2.0-29.7); median number of apheresis 1 (range 1-3); same efficiency with each program. After conditioning regimen (BEAM, BuCy, HD melphalan) time to hematopoietic recovery was adequate: median time to ANC > 0.5×10^9/L 10 days (range 8-12); median time to Plt > 50×10^9/L 12 days (range 8-14). Either infection nor organ toxicities > 2 (WHO) occurred. This single centre experience underscores feasibility of autologous PBSC collection in unfavorable cohort of patients requiring eradication therapy. Adjusted apheresis procedures should be evaluated, in agreement with patients characteristics at onset and response to chemotherapy.

PUB10
HEMATOLOGICAL TOXICITY OBSERVED WITH THE USE OF BUSULFAN IN THE CONDITIONING REGIMEN FOR PERIPHERAL BLOOD STEM CELL TRANSPLANT: CASE REPORT

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Busulfan (BU) is an alkylating agent employed in the treatment of many hematologic diseases. High doses BU is an highly toxic chemotherapeutic regimen, mostly acting on the hematopoiesis, and widely used in association with other drugs as conditioning regimen for bone marrow transplantation. Busulfan toxicity is dose-dependent and generally reversible, but in some cases, quite often after overdose, an irreversible toxicity has been shown. At our Institution, BU in association with Melphalan (L-PAM), is employed as conditioning regimen in acute myeloid leukemia (AML) patients who underwent autologous bone marrow transplantation (ABMT). In this study we report an unusual case of severe hematological toxicity observed in a AML patient undergone ABMT. M.M., a patient with a diagnosis of AML-M2, achieved complete remission after induction therapy consisting of idarubicin, cytosine arabinoside, etoposide consolidated by high doses of Ara-C and MEC regimens followed by peripheral blood stem cells collection. After two months from the end of the therapy, the patient relapsed. An ABMT was then performed after myeloablative therapy consisting of BU (240 mg/die x 4 days) and L-PAM (200 mg). The patient developed a severe and prolonged hematological toxicity requiring platelets and red blood cell units transfusions extensively, finally evolving to a myelodysplastic syndrome after seven months. BU levels in plasma and in leucocytes were measured with HPLC. BU levels increased soon after the first dose, and reached the highest level at day 3 and 4 after treatment. At the end of the treatment, we observed a slowly decrurent of BU plasma concentration, still present at lower levels at day 8 (+1 from reinfusion of PBSC). We also measured BU levels in the intracellular compartment, showing that this compound has a slower intracellular kinetic compared to the plasma kinetic, eventhough we found higher concentrations in our patient leucocytes compared to those reported in other patients. Analysis of our data suggests that the prolonged hematological toxicity observed in this patient can be caused by a stem cell damage due to the high sensibility and persistence of BU in the blood at the moment of reinfusion.
ORGANIZATION OF TRANSFUSION THERAPY AND EVALUATION OF LIFE QUALITY IN PATIENTS AFFECTED BY CHRONIC HEMATOLOGY DISEASES

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The Service of Immunohematology and Transfusion (SIT) of Naples’ University “Federico II” began practising transfusion therapy in 1994, especially in collaboration with the department of Hematology, to respond to a double demand from both patients and departments. The patients, who after they have received a diagnosis, need a transfusion therapy, that should be practised at home, in the original department or in other city hospitals. For them there is the advantage that the SIT offers qualified, punctual treatments and at the same time in tune with the original departments treatment, responding to logistic, safety and assistance demands. For the department it solves the problem of relief stoppage, due to the big number of patients, and manifold therapeutic activities that take place. Carrying out the transfusions in the SIT reduces the relief stoppage. Moreover, therapeutic strategies in the management of the patients with chronic pathologies that need periodic transfusions, change with time, imposing a hypertransfusion regimen to assure the best life quality possible. It is, in fact, more and more appreciated the demand to consider the effectiveness of a therapy, not only to the gain of survival but also considering the health general conditions of the patient. Life quality is an aspect difficult to appraise, because it presupposes judgments, experiences and evaluations that can be different from person to person. We have formulated a questionnaire for our patients to appraise their life quality. The questions concerned their clinical and psycho-physical conditions using a simple and comprehensible terminology for all patient. The answers of the questionnaire have been appraised according to the patient’s age and the existence of concomitant pathologies. Currently around 36 patients come to the SIT monthly, with chronic hematology disease: refractory: treatment was discontinued after 6 doses (plts=2×10^10/L). Up to now, she was given IV. HDIgG obtaining a C.R. In our experience, i.v. anti-D Ig, were effective in 2/3 treated patients; even if small numbers, the response seems to be no related to response to i.v. HDIgG. No side effects were recorded.

SEVERE JUVENILE VAGINAL BLEEDING DUE TO GLANZMANN’S THROMbasthENIA. CASE REPORT

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The prognosis of patients with thrombasthenia is good, although the severity of bleeding is unpredictable. Acute juvenile vaginal bleeding is one of the important clinical manifestations of this disorder which sometimes necessitates aggressive and invasive treatment. We describe the clinical course and management of a 14-year-old girl with Glanzmann’s thrombasthenia. Case Report. A 14-year-old girl was admitted to the Gynecology Department due to acute menorrhagia during her first menstrual period. She was known to have Glanzmann’s Thrombasthenia since 8 years old. She had been hospitalized in the past due to nasal bleeding, which required often nasal packed. The first menstrual period began 2 weeks before hospitalization and lasted until the day of her admission. Physical examination revealed ecchymosis to the legs. Gynecological examination by speculum showed heavy bleeding. Laboratory findings showed a hemoglobin of 5.8 g/dL and reticulocytosis with no evidence of hemolysis. Platelet count, prothrombin time (PT), and partial thromboplastin time (PTT) were normal. The bleeding time performed by the Ivy method was 12 min. The vaginal bleeding gradually worsened and the concentration of hemoglobin decreased to 4.5 g/dL. The patient received 3 U of packed red blood cells. Treatment with oral Tranexamic acid 500 mg three times daily and conjugate estrogen tablets 1.25 mg daily was instituted. The vaginal bleeding did not subside, requiring treatment with fresh frozen plasma (3U) and platelets (4U). Abdominal ultrasound showed hematoculopus and hematometra. Examination under general anesthesia was performed, and fresh blood was seen emanating from the uterine cavity. An uterine tampon...
was inserted and was able to tamponade the bleeding. The intrauterine tampon was removed 48 hr later and no active bleeding was evident. The patient was discharged after eight days and oral contraceptive was initiated. After seven months the patient was well and the blood menstrual was normal. We concluded that although thrombasthenia is a rare disorder, it should be considered in diagnosis of young women presenting with abnormal menstrual bleeding.

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Pulmonary thromboembolism (PE) is easily detected by spiral CT scan which collects data useful to rebuild scan frames in a short time (1 second for layers of 2 mm) necessary to minimize body movements. Thirty rotations of X-ray tube and detectors acquire the pulmonary arteries volume during the highest concentration of iodate contrast medium (icm). In this way PE is showed by strong icm enhancement difference with respect to vasal lumen. We used Hitachi radix spiral CT scan, model Prima turbo, with a Silicon Graphix workstation and software VITREA which allows to be rebuilt images on different spatial planes, in 3D too. We have to examine the thorax from the aortic arch to pulmonary arteries. Apnea is necessary, with collimation 3 mm, pitch 1.5-2, table feed velocity 5-6 mm per second, reconstitution index 1.2 mm. A total of 120-150 mL of non-ionic icm is injected in an arm vein in concentration of 300-350, flow 4-5 mL per second; delay is well defined by a scan test with a spot test image. Ten patients we suspected had pulmonary embolism were studied (six showed proximal deep venous thrombosis of the legs according to compression ultrasonography scan); five were affected by proximal pulmonary thromboembolism, four by segmentary thromboembolism and three resulted negative. Limit of experience demonstrates that spiral CT scan, used by skillful operator, allows pulmonary thromboembolism to be diagnosed easily.

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Genetic predisposition to hematic hypercoagulability is well known long since. Antithrombin deficiency and disfibrinogene- mia were the first inherited thrombophilias to be described, later were identified heterozygous deficiencies of protein C and S; nevertheless these were the cause of only a few cases of idiopathic thrombosis. The state of knowledge changed remarkably with the discovery of resistance to activated protein C (1993) and mutation of prothrombin (1996). The resistance to activat- ed protein C results from the substitution of adenine for guanine at nucleotide 1691 of the factor V gene (G1691A), which caus-
ence were found in the percentage of abnormal vs normal metaphases in each abnormal case. We also evaluated the quality of the cells giving a score 1 + to 3+ (3 meaning best cells). We found a better quality of metaphases in the +5637 cultures. In conclusion we did not find differences with or without the use of the 5637 CM in our series of samples, except for a slightly better quality of cells.

PUB17
A CASE OF i(11)(q10) AS SOLE ABNORMALITY IN A PATIENT WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Recurring translocations involving 11q23 are of great interest in acute leukemias and the MLL (Myeloid lymphoid leukemia) gene has been found rearranged with more than 40 different partner genes, 20 of which have been cloned. The result of the translocation is a chimeric gene on the rearranged chromosome 11 consisting of the 5' region of MLL and the 3' region of the partner gene. A gain of chromosome 11 is a recurring abnormality found in AML patients as sole abnormality. Only few cases with +11 have been described in thought in ALL as sole abnormality in the literature, and just one to our knowledge as i(11q). Here we report an interesting case of a 65 years old female patient presenting with WBC 24.4 × 10^9/L, Hb12.9 g/dL, PLT 245. × 10^9/L. Analysis of the bone marrow aspirate led to a diagnosis of acute lymphoblastic leukemia (ALL). The immunophenotype of the leukemic cells showed: CD19+, CD79a+, TdT+, CD10+, CD5+, CD13+, CD33+, compatible with an early pre-B ALL, coexpressing myeloid antigens. Cytogenetic analysis of metaphase cells obtained from 48 hours cultures, showed a karyotype with: 46,XY, t(20;21)(q12;q22.3), t(9;22)(q34;q11)[19] 47,XY, t(20;21)(q12;q22.3), t(9;22)(q34;q11), +der(22)(q22) (q34;q11)[11]. This is the first time that this translocation is described in the literature, to our knowledge, the major point of interest being the possible involvement of ALL1 with an unknown gene at 20q22. ALL1, which encodes a nuclear transcription factor (TF) shows homology in its 5' part with the Drosophila melanogaster segmentation gene, runt, and contains a transactivation domain in the carboxyterminal portion, involved in many recurring translocations in leukemias. The patient is currently under treatment with STI 571 waiting for allogeneic bone marrow transplantation.

PUB19
A CASE OF T-CELL PROLYMPHOCYTIC LEUKEMIA WITH GOOD RESPONSE TO PENTOSTATIN

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T cell prolymphocytic leukemia (T-PLL) is a rare malignancy of post-thymic T-cell, with distinctive biological and clinical features. The treatment is difficult both for the rapid aggressive clinical course and for its refractoriness to conventional therapy used in lymphoid disorders. In January 2001 a 70 year old man, admitted to our department, was diagnosed a small cell T-PLL (FAB classification). Immunological markers showed a T-PLL phenotype: TdT+, CD2+, CD3+, CD5+, CD19, CD4+, CD3α+, CD3β+ and CD16-. The karyotype analysis failed. The clinical features at the onset were: organomegalias (lymphonodes, spleen and liver enlargement), a raised lymphocyte count (200 × 10^9/L) and a significant renal failure (Creat 3 mg/dL). The lymphocytes rose in spite of the therapy (fludarabine, cyclophosphamide, methotrexate) to 300 × 10^9/L. On consulting previous case-reports a therapy with pentostatin was started in day hospital at a dosage of 4 mg/m^2 weekly for 4 weeks, then every 14 days for 4 weeks and then monthly. After 3 months of therapy the patient was in a very good condition, with no renal failure, decreasing number of lymphocytes (40 × 10^9/L), reduction of over 50% of organomegalias and no need for transfusion therapy. As in the few other known cases pentostatin has proved to be a very active drug with no toxicity and feasible in day hospital: a longer follow-up is however necessary to define doses and duration of the maintenance therapy.
COMPLICATIONS OF AB0-INCOMPATIBLE BONE MARROW TRANSPLANTATION

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74 pts underwent HLA-matched non-T-cell-depleted BMT between June 1993 and December 1999. 20 pts (Group 1 = 9 M, 11 F; median age 34 yrs, range 20-51) were AB0 major or major/minor mismatched (ratio MUD/Sibling = 6/14), 15 pts (Group 2 = 7 M, 8 F; median age 40 yrs, range 24-51) AB0 minor mismatched (ratio MUD/Sibling = 6/9), 39 pts (Group 3 = 20 M, 19 F; median age 36 yrs, range 16-55) were AB0 matched (ratio MUD/Sibling = 8/31). 72 pts received donor marrow and 2 pts AB0 matched peripheral blood stem cells. Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine and methotrexate. To prevent hemolytic complications we used the following procedures: plasma removal by marrow centrifugation (setting AB0 minor and major/minor mismatched), red cell removal by differential centrifugation and/or plasma exchange, when the IgG/IgM isohemagglutinin titers were above 1:16 (setting AB0 major mismatched). All pts had a complete engraftment; however on day 20 post-BMT 3/20 pts (Group 1), 2/15 pts (Group 2) and 21/39 pts (Group 3) (p=0.004) reached a neutrophil counts above 0.5×10^9/L. No differences were observed in red cell and platelet transfusion requirements (p>0.3), nor in acute GVHD, transplant related mortality (TRM) and hemolytic complications incidence (p>0.3) among the three Groups. Our results showed a faster rise of neutrophil counts in the setting AB0 compatible; nevertheless AB0 incompatibility did not influence transfusion requirements nor the incidence of TRM, GVHD and hemolytic complications.

EPIDEMIOLOGY OF ACUTE LEUKAEMIA IN CALABRIA

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Recently, media have claimed about an increase of acute leukemia (AL) risk in our region mainly due to exposure to stronger magnetic fields. Our objective was to investigate whether the observed incidence and geographical distribution confirm or reject this hypothesis. Two sources of data were used: 1) a computerized file containing hospital-discharge abstracts, with diagnoses and procedures for all the hospitalizations in Calabrian residents; 2) a computerized data base maintained by Regional Hematological Registry Calabria, an Italian region with 2.070.203 inhabitants. In 1997-1998, 280 new cases of AL with an annual standard rate (SR) of 8.46 per 100.000 inhabitants were diagnosed. The AL incidence in our region, in which Agriculture and Services are the main occupations, was quite similar to the other regions of Southern and Middle Italy. The geographical distribution of the disease was highly variable with higher SD in countries in which population overcome 10,000 inhabitants. Compared with the other countries classified on the basis of economic activities, rural countries were at lower risk of AL (SR 5.97). On the contrary, AL was more common among the inhabitants of industrialized countries. There were notable differences in age distribution by subtype. The highest incidence rate of AL was observed in the population who were >64 years old. Acute Lymphoid Leukemia (ALL) was registered in 127 patients (SR 3.73) - 54 were children, 40 were in age group between 15 and 64 years and 33 were >64 years old. In contrast, Acute non Lymphoid Leukemia (ANLL) was prevalent in older people. In conclusion, the incidence distribution of AL for Calabria is quite similar to the typical pattern previously described. No excess of incidence justifies the hypothesis of an increasing risk of acute leukemia in our region.

GRAFT-VERSUS-HOST DISEASE AND CYTOMEGALOVIRUS PREVENTION IN PATIENTS UNDERGOING BONE MARROW TRANSPLANT FOR HEMATOLOGICAL MALIGNANCIES WITH THE DEPLETION OF LEUKOCYTES BY FILTRATION OF BLOOD PRODUCTS

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The prevention of GVHD in immunocompromised hosts for TMO in the course of hematologic disease and submitted to transfusion is generally performed by irradiation of blood products with 25 to 40 Gy; the prevention of CMV infection is also performed with the irradiation of blood products or by choosing seronegative donors. Alternatively the use of filters for depletion of leukocytes is considered effective in the prevention of CMV infection and presumably the GVHD. We present our experience in a cohort of high risk patients for GVHD and CMV incidence who received TMO mostly for hematologic malignancies with filtered blood products instead of irradiated products. Among 30 patients who received TMO during 1999 and 2000 22 patients are considered evaluable for acute GVHD and CMV infection with minimum two months follow-up. The mean age of patients was 50y (range 14-66y). Among evaluable patients we have Acute Leukemia (9 pts with 5 high risk AM L and 4 high risk ALL), resistant-relapsed lymphoma (6 pts), secondary myelodisplastic syndrome (3 pts) resistant multiple myeloma (2 pts) and severely pretreated hystiocytosis X (1 pt). The mean number of transfusion of RBC was 4 units (range 0-12) and of PLT was 6 (range 1-16). GVHD severe (grade III-IV) was recorded in 10% of patients with more advantage age; GVHD mild was also recorded in older patients without specific connection with the type of disease. CMV infection was recorded in 32% of patients with clinical evidence in 14% of patients in which the event was particularly severe with the death of patients; however the infection was recognized later between 3 and 12 months after TMO. The CMV positivity was recorded exclusively in patients older than 50y and almost only in lymphoproliferative diseases, mostly lymphomas. The depletion of leukocytes was documented in a series of RBC and PLT units as more than 4 log from the initial numbers. In conclusion the depletion of leukocytes by fil-
Cutaneous involvement in a IgG/λ multiple myeloma

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Multiple myeloma is a disease caused by neoplastic plasma cells that synthesize abnormal amount of immunoglobulins. Cutaneous manifestations of multiple myeloma are very uncommon. Skin can be involved either as a direct extension of underlying medullary disease or, rarely, as a result of hematogenous spread. We described a 77-years-old female presented on 1995 a monoclonal gammopathy of undetermined significance (MGUS). On 1999, four years after diagnosis, serum protein electrophoresis indicated a marked increase of monoclonal IgG lambda paraprotein with suppression of the other immunoglobulin classes. Bone marrow biopsy revealed the presence of abnormal plasma cells at the rate of 40-50% of all nucleated cells. Further investigations did not show osteolytic lesions or renal failure. The patient was treated with six courses of chemotherapy according to the MIP schedule (melphalan, idarubicine and prednisone) between May and October 1999. The IgG level fell and the bone marrow reassessment showed a rate of plasma cells lower than 5% of nucleated cells. Between October 1999 and May 2000 she received interferon (1.000.000 U three time a week) until relapse occurred with an increase of IgG up to 4 g/L and plasmacellular bone marrow infiltration over than 80%. The patient received five courses in accord to MP schedule (Melphalan, Adriamycin, Dexamethasone) with a very good clinical response of the skin lesions.

An hematologist has no doubt in associating the contemporary presence of monoclonal gammopathy, renal failure and hypercalcaemia with the diagnosis of multiple myeloma. In March 2000 we saw a patient, 48 years old employed as a teacher, sent by her practitioner suspecting a myeloma. In 1984 she underwent thyroidectomy for papillary carcinoma (therapy with levothyroxin), in 1998 hysterectomy for uterine leiomyoma; from 1999 she was treated with ACE inhibitors for hypertension. She presented an elevation of γ-globulins (21%) with a IgG monoclonal component of k type of 15.7 Gm/L, urine protein 0.1 Gm/24h, serum calcium (12.4 mg/dL–3.4 mmol/L) and serum creatinine elevated (3.1 mg/dL–274 umol/L). Peripheral blood counts were normal and the patient had no bone pain. A following check-up did not find light chains in the urine neither lytic bone lesions. At this stage it was clear that renal failure and hypercalcaemia were non correlated with monoclonal gammopathy. Kidney ultrasonography showed the images of nephrocalcinosis with hyperecogenic pyramids owing to salts deposition, multiple papillary calcifications and dilated distal collecting ducts with little stones. A careful examination of patient’s story could explain the case: from the time of the thyroidectomy she continued in taking vitamin D2 (ergocalciferol) at a daily dose of 0.5 mg, prescribed to prevent a possible hypocalcaemia. The drug caused an hypercalcemic-hypercalciuric syndrome which underlines as an apparently simple diagnosis sometimes can hide a more complex pathology, fortunately this time less serious and dangerous.

Thalidomide-based treatment for advanced multiple myeloma

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The role of angiogenesis in neoplastic diseases is under investigation since many years; recently, because of discovery of regulator factors of angiogenesis and because of availability of their inhibitors, a clinical interest has also emerged. Increased production of angiogenetic factors (VEGF, FGF), has been shown in various diseases of hematological interest, especially in multiple myeloma. Thalidomide is a drug with well documented antiangiogenetic activity and with in vivo good tolerance. From June 2000, we treated with thalidomide four patients suffering from multiple myeloma (MM), refractory to multiple lines of...
therapy (MP, VAD etc.), two of whom previously transplanted and relapsed. All patients were males, with a median age of 62 years; thalidomide was given at the initial dose of 200 mg/day, with weekly increase of 100 mg/day until the highest dose of 600 mg/day or until appearance of severe side effects. Only one patient reached 600 mg/day; a marked constipation in three patients did not allow to overtake 400 mg/day (one patient), or 200 mg/day (two patients). In three patients thalidomide was given with fortnightly pulses of dexamethasone (20 mg i.v. days 1-4 and 15-18). In three patients there was a decrease of the monoclonal component (MC) > 50%, with a median time to response of two months (the fourth patient has just started the treatment). In three patients we noted improvement of the hemoglobin level (increased of almost 4 g/dL). Bone marrow aspirate performed in two patient before and during treatment did not show significant reduction of the plasma cell infiltration; the only side effect manifested was constipation; in 2 patients we noted eosinophilia (496/mmcc and 352/mmcc respectively). All patients are alive, the clinical status is excellent and improved in respect to pretreatment. In conclusion, thalidomide seems to be effective in the treatment of patients with refractory MM. The mechanism of action is still uncertain. Probably it has multiple actions: inhibition of regulatory factors of neoangiogenesis; direct inhibition of growth of tumor or stromal cells; modification of the interaction stroma/plasma cell; alteration of adhesion molecules; modulation of cytokine production, by reducing those that improve tumor growth (eg. IL-6) and/or by increasing inhibiting cytokines (eg. IL-2 and IFN). Further biological and clinical studies are needed to assess the role of thalidomide in MM, in order to plan the use of other antiangiogenetic drugs and clinical studies are needed to assess the role of thalidomide in MM, in order to plan the use of other antiangiogenetic drugs and to evaluate the efficacy of these drugs in combination with chemotherapy in early stage MM patients.

**SUCCESSFUL TREATMENT BY A COMBINED REGIMEN WITH METHOTREXATE AND PREDNISONE FOR MULTICENTRIC CASTLEMAN’S DISEASE AND AILD-TYPE T-CELL LYMPHOMA**

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Multicentric Castleman’s disease (MCD) and angioimmunoblastic lymphadenopathy with dysproteinemia (AILD)-type T-cell lymphoma are uncommon atypical lymphoproliferative disorders of uncertain cause characterized by almost identical clinical and biological signs. Patients frequently have systemic manifestations such as fever, generalized lymphadenopathy, hepatosplenomegaly, skin rash, anemia, hypogammaglobulinemia, hypoalbuminemia, and an increase in acute phase proteins. Most observations suggest that MCD may be a disorder of the immune system, usually considered as benign lymphatic hyperplasia with a chronic course. However, the long-term development of lymphoma is under question, because of some analogies between MCD and AILD in which lymphomatous transformation has been described. Although both diseases share clinical signs, they show different morphologic patterns in lymph node and in some cases an intermediate picture has been reported. These disorders are often refractory to treatment even with corticosteroids or chemotherapy, and consequently the prognosis for such patients is poor. Infections are a common cause of death. Due to these findings, we have used a combination of oral methotrexate and prednisone as first line treatment in three cases of MCD and, on the other hand, in two patients with refractory AILD-type T-cell lymphoma. Methotrexate is a folate antagonist extensively used in GVHD, rheumatoid arthritis and other chronic inflammatory disorders. Methotrexate was administered orally as low-dose (10 mg/m² once weekly). The patients did not received other treatment with the exception of prednisone (15 mg/day tapered down to 5 mg/day). The exact mechanism of immunosuppressive and cytoreductive properties associated with low dose methotrexate is still elusive. It has been reported that it is an effective treatment for some patients with LGL leukemia. We achieved a dramatic prolonged improvement of the systemic signs and disappearance of the tumor burden in the AILD-type T-cell lymphoma and a satisfactory and prolonged control in MCD. Treatment was well tolerated. We believe that methotrexate-based treatment can represent a potential new therapy based on the pathophysiological mechanism of these disorders, which despite the aggressive systemic therapy have a poor outcome.

### Main clinical and laboratory findings of patients at diagnosis.

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**NON-HODGKIN’S LYMPHOMA OF THE ORAL CAVITY: REPORT OF FOUR CASES**

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Extranodal non-Hodgkin’s lymphomas are relatively uncommon comprising about 20% of all the cases reported. Within these the occurrence in the oral cavity comprised from 1 to 2% of the cases in literature. The incidence of the presenting site being the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower. For this entity there are only individual cases in recent literature. From November 1999 to May 2000 to Division of Hematology we observed 4 males patients with mean age 54 years (range 79-44 years) whose received a diagnosis of NHL of the oral soft tissues. The initial clinical symptoms were: enlargement of the oral soft tissues, that involve generally the gum, is still lower.
The laboratory data were all normal (including markers in the serum involved more frequently in lymphoproliferative disorders). All patients were submitted to local biopsy whose histological examination concluded for diffuse large B-cell lymphoma (B-DLCL) of the REAL classification. The staging for images and the bone-marrow biopsy identified 3 patients in stage IEA and 1 in stage IV. All the patients were treated with polychemotherapy (3 MACOP-B and 1 CHOP). All patients achieved complete remission, maintained to all today, with mean follow-up of 15 months (range 12-20 months). The NHL of the oral cavity soft tissues are very rare nosological entity, the clinical symptoms to the presentation often misdiagnosed for other more common oral disease, for which the diagnosis is very difficult at first examination. Routine treatment guidelines don’t exist because in the literature there are only individual cases. Rarely it’s used radiotherapy only (40-50 Gy for 4-6 weeks) or combined with administration of anti-tumour single agents (6MP-PDN-CTX). Our choice of aggressive treatment with doxorubicin has been made in consideration of the aggressive histological type, the good performance status of the patients, according also to the modern literature for extra nodal diseases.

**PUB28**

HHV8-ASSOCIATED MULTICENTRIC CASTLEMAN DISEASE: A RAPIDLY FATAL DISEASE WITH EVENTUAL PROGRESSION TO PLASMABLASTIC MICROLYMPHOMAS. REPORT OF 2 CASES

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The Human Herpes Virus 8 (HHV8) has been associated to Kaposi sarcoma (KS), and to AIDS-related primary effusion lymphoma (PEL). Moreover, HHV8 sequences have been found in some atypical lymphoproliferations such as multicentric Castleman’s disease (MCD). Two cases of MCD, plasmacellular type, occurred in our Institution in male patients, aged 74 and 54, over a period of 14 years (1984-1998). Polyadenopathy and polyclonal hypergammaglobulinemia were the presenting symptoms in association with severe, transfusion-insensitive, anemia, with autoimmune features and hemolytic progression. The patients worsened very rapidly, refractory to treatment, and died in about one month. In the 2nd (HHV-Ab negative) case, a disseminated intravascular coagulation was found at autopsy. We found by immunohistochemistry HHV8+ scattered cells and by polymerase chain reaction HHV8 sequences in lymph nodes in both cases. In the 2nd case, lambda-restricted IgM+ plasmablasts, HHV8+, were seen in mantle zone in a first biopsy, and coalesced in plasmablastic, germinal center-replacing, microlymphoma in a 2nd biopsy performed few days later. By in situ hybridization Epstein-Barr virus was negative in both cases. Our data confirm the HHV8 association with MCD and support the described HHV8 role as lymphomagenic agent.


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A primary muscle NHL is an extremely rare event: the muscle groups more frequently involved, especially in elderly patients, are the muscles of the extremities and of the pelvis, rarely those of the head or neck. Generally the muscular NHL presents a phenotype to B cells, an aggressive course and a poor prognosis. We report here the case of a NHL of the muscle deltoid as a secondary neoplasm of preceding lymphoproliferative disorder. M.P., a man of 64 years, had a prior diagnosis of HCL, formulated in March 1988. For this pathology the patient had received therapy with: a) 1 INF cycle (March 1988–March 1989); b) deoxycytomycin (DCF) (April 1990–March 1991); c) DCF+INF (October 1997–October 1998). In March 1999 the patient had NMR imaging for the appearance of a voluminous swelling, strongly aching, in the right shoulder. The radiological image showed an infiltrated muscle, isointense on the T1-weighted sequence and hyperintense on the fast spin-echo T2-weighted sequence, with varying enhancement after dye. The neoformation involved the deltoid muscle, the soft contiguous tissues and the head of the humerus. The muscle biopsy, as well as the bone biopsies, put in evidence a picture of infiltration of large cells with lymphoid pattern (CD 20+, +KBS+, BCL2+, negative UCHL-1). There was no evidence of infiltration by hairy cells. On the basis of these results a diagnosis of large cell NHL, B phenotype, stage IV, was formulated. The patient was subjected to chemotherapy according to protocol P-VABEC getting a CR ailing about one month. Before begin the radiotherapy of consolidation the patient presented a relapse of disease so was subjected to chemotherapy with high dose cyclophosphamide + local radiotherapy. After this second approach the patient had a partial response whose duration has been some months. The incidence of secondary neoplasm in the patients affected by HCL, is in different series, variables, from the 5% at 13%; is not still definite good if the immunosuppression, peculiar of the HCL, as well as the induced one from the therapy with analogous of the purine, play a role conclusive in the development of the secondary neoplasm. In our case-report this is the only patients that developed a LNHL as secondary neoplasm. Also in this case isolated muscle lymphoma has proven a clinical entity with an aggressive course and a poor prognosis. Probably for this type of NHL high dose chemotherapy as first line treatment is the best therapeutic approach.

This report describe a case of pulmonary Lymphomatoid Granulomatosis (LG) associated with monoclonal gammopathy (MG) evolving to Lymphoma. BS, a 64 years old man, was admitted to our Dept. of Medicine for dyspnea, cough and fever. The past medical history of the patient was negative. There was no history of hereditary illness and use of alcohol, tobacco and drugs. The physical examination was negative but chest X-Ray showed a diffuse interstitial reticulo-nodular infiltration. The usual routine biochemistry was normal except a relative lymphocytosis; there was an increased of serum IgM component less than 3 g/dL. The patient was transplanted and monoclonal gammopathy (MG) was detected. The serum immunoglobulins electrophoresis showed a double component, peculiar of the HCL, as well as the induced one from the therapy with analogous of the purine, play a role conclusive in the development of the secondary neoplasm. In our case-report this is the only patients that developed a LNHL as secondary neoplasm. Also in this case isolated muscle lymphoma has proven a clinical entity with an aggressive course and a poor prognosis. Probably for this type of NHL high dose chemotherapy as first line treatment is the best therapeutic approach.
was treated with antibiotic therapy without improvement of clinical condition. Pulmonary HR-CT Scan confirmed the pattern of interstitial multiple nodules and pulmonary biopsy showed an angiocentric and angiodestructive, polymorphic granulomatosis. This histological picture led to the diagnosis of LG. The presence of cytological atypia suggested an evolution into B-cells Lymphoma. LG is an angiocentric lymphoid immunoproliferative lesion involving the lung especially. The pulmonary infiltrates are polymorphous with lymphoid cells with or without atypical cells and angioinvasion; the presence of polymorphic lymphoid infiltrate composed of small lymphocytes, plasma cells, and variable numbers of large atypical mononuclear cells is a typical feature. Tumor-like nodules with central necrosis are present sometimes. LG evolves into lymphoma in the 50% of cases. Hystopathologic findings of LG are also present in several disease processes as cancers, autoimmune-type diseases and infections. Laboratory data were not particularly helpful in establishing the diagnosis of LG: the erythrocyte sedimentation is high and there is a relative monocytosis. Frequently the concentration of Ig is increased. Some cases of association of LG and MG with secondary evolution into pulmonary lymphoma, representing an epiphenomenon is presented in the literature. In 50% of cases LG and MG could be present in association.


SPLENIC HAMARTOMA MIMICKING SPLENIC NEOPLASM DIAGNOSED BY SPLENIC PERCOUTANEOUS GUIDED BIOPSY

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A 16-year-old man presented with abdominal trauma to a local hospital. Physical examination was normal. Ultrasonography (US) of the abdomen revealed a single rounded, predominantly hypoechoic lesion of the spleen 2.5 cm in diameter. The spleen’s diameters were within normal limits. A computed tomography (CT) scan of the abdomen confirmed the hypodense lesion within the splenic parenchyma. The liver, kidneys and pancreas were normal and no abdominal lymphadenopathies were detected. A CT scan of the chest was normal. Primary splenic lymphoma (PSL) and metastatic tumor were considered in the differential diagnosis. Other imaging techniques such as magnetic resonance imaging (MRI), scintigraphy with gallium and technetium did not help in the differential diagnosis. The patient underwent US-guided fine needle aspiration biopsy of the splenic lesion at a local hospital but cytological specimens were not diagnostic. He was then admitted to our department and underwent US-guided tissue core biopsy of the splenic lesion. Histopathologic examination of biopsy specimen revealed a disorganized series of anastomotic channels lined and supported by endothelial-type cells consistent with splenic hamartoma. The patient was discharged and he is well and in good health two months after the splenic biopsy. Splenoma or splenic hamartoma is a rare primary splenic tumor often incidentally discovered at laparotomy or autopsy. Hamartoma of the spleen are usually asymptomatic and there are a few reports of sympto-
Diagnosis appears to be a major factor causing poor outcome. Late picture was observed after salvage chemotherapy DHAP followed by Rituximab and MINE failures. The patient showed an unusual cardiac localization in PCL patients; myocardial tissue and thoracic wall. No changes of the clinical thorax. The CT revealed a new retrosternal mass infiltrating the pleural mass and the biopsy revealed a large B cell lymphoma arising from germinative centers (CD20+, CD79a+, CD123+, CD61+, CD45RO+, CD74). Total body computed tomography scan (CT), and bone marrow biopsy demonstrated no other sites of disease. CHOP chemotherapy for 8 courses was applied obtaining a major reduction of the mass. No signs of hemodynamic compromise were observed during treatment.

The PCL is defined as a lymphoma involving only the heart and/or pericardium. PCL is a rare entity in immunocompetent patients and is associated with poor prognosis. Variability of clinical presentation and atypical symptoms may cause a diagnostic delay. More than 80% of cases reviewed in literature in immunocompetent patients were diffuse B-cell lymphomas (mainly large-cell type) usually arising in the right chambers. Case Report. Thirty-four year-old man with a recent history of dyspnea and precordial pain. Previous abuse of drugs and no history of cardiovascular diseases were reported. A chest X-ray revealed cardiomegaly while inverted T waves appeared on an electrocardiogram. Trans-thoracic echocardiogram revealed small pericardial effusion and hypocoysinetic apex. Lactate dehydrogenase was slightly elevated. The patient was negative for HIV, positive for HCV and HBV without signs of active hepatitis. Diagnosis of an ischemic damage complicated with viral pericarditis was made. Further serials trans-thoracic echocardiograms were performed showing a rapidly expansive mass. Magnetic resonance imaging revealed a mass measuring about 5 cm. Infiltrating left cardiac apex and pericardium reducing the contractile myocardial function. Moreover there were other multiple nodules measuring from 1.5 and 4 cm in right ventricle. The thoracotomy confirmed the presence of the transmural myocardial mass and the biopsy revealed a large B cell lymphoma arising from germinative centers (CD20+, CD79a+, Bcl2+, Bcl6+, CD10+, CD45RO+, CD74). Total body computed tomography scan (CT), and bone marrow biopsy demonstrated no other sites of disease.

Purpose. The aim of this study was to investigate the effectiveness and the efficacy of CEOP-VP16 chemotherapy regimen in patient with high grade non-Hodgkin’s lymphoma in peripheral Hematology/Oncology Units. Patients and Methods. Between 1994 and 1998, sixteen patients aged 28-72 years old with high grade non-Hodgkin’s lymphoma were enrolled onto our study; eligible patients were 18-75 years old with B or T cell high grade non-Hodgkin’s lymphoma. Exclusion criteria: prior chemo/radiotherapy, HIV positivity and primary CNS disease. Patients were treated with CEOP-VP16 regimen (CTX 750 mg/m2 day 1, EPI 90 mg/m2 g 1, VP16 100 mg/m2 days 1-2-3, Prednisone 100 mg days 1-2-3-4-5) for six cycles every four weeks. Results. Complete and partial response rates were 80% (12 patients) and 13% (2 patients) respectively, with an ORR of 93%. One patient died during chemotherapy; it wasn’t possible to determine the cause of death because the relatives refused the autopsy. After a median follow-up of 48 months, 3 relapses, at 12, 18 and 24 months after the end of chemotherapy, occurred in CNS, abdominal lymph nodes and abdominal lymph nodes with bulky disease; the first two patients were treated with salvage chemotherapy, achieving PR, the last one was treated with high dose chemotherapy and hematopoietic stem-cell transplantation, achieving CR. The 3-years OS is 93%. The treatment, despite the significant hematological toxicity, was well tolerated. Grade 3 alopecia developed in all patients. All experienced grade 4 neutropenia (that suggested the use of G-CSF to reduce the period of neutropenia) with 60% of febrile patients, successfully cured with antibiotic therapy. Thirteen patients (86%) developed oral candidiasis. No other grade 3-4 toxicity was reported. Conclusions. CEOP-VP16 is a feasible treatment, with significant efficacy in high grade non-Hodgkin’s lymphomas, cured in peripheral Hematology/Oncology Units. The ORR is high (93%) as reported in other studies. CRs are durable but a longer follow-up and an higher number of patients are warranted to confirm the efficacy of this protocol.
round and infiltrated by a whitish, rubbery tissue all along its course. The histologic findings were consistent with high grade diffuse large cell lymphoma of peripheral B lymphocyte origin, primarily arising in the saphenous vein. The patient is alive and tumor free 16 months after the operation and six courses of chemotherapy (CVP). To the best of our knowledge there are no other reported cases of lymphoma arising in saphenous vein.

**PUB38**

APPEARANCE OF A LYMPHOPROLIFERATIVE B DISEASE IN A PATIENT PREVIOUSLY TREATED FOR T CELL LYMPHOMA

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On may 1988 a 45 years old man was diagnosed as having a pleomorphic medium and large T cell Lymphoma, according to Kiel classification, by an inguinal lymph node biopsy. Clinical stage was IV A because of the presence of abdominal lymph nodes and a moderate bone marrow infiltration. He was treated with MACOP-B regimen of chemotherapy and reached a good partial remission of abdominal disease (≥90%), with a negative bone marrow biopsy. On April 1989 he had an abdominal, inguinal and axillary progression of disease. A new lymph nodal biopsy was performed and the diagnosis was consistent with pleomorphic small T cell lymphoma. The patient was treated with CHOP regimen for 4 cycles and reached a complete remission, that lasted until may 1999. In that period axillary and neck lymph nodes appeared and a leucocytosis with absolute lymphocytosis (12.8×10⁹/L) broke out. A cytofluorimetric assay showed they were B cells expressing CD5 and CD19 markers. On June 1999 a CT scan showed multiple enlarged abdominal lymph nodes extended until the pelvic region. A lymph node biopsy was performed and it was consistent with the diagnosis of small lymphocytic lymphoma or B-lymphocytic CLL according to REAL classification; molecular biology assay showed a monoclonal rearrangement of immune globulin heavy chain gene. A bone marrow biopsy was also performed and it showed a diffuse infiltration of B cells CD5 and CD20 positives. Clonality of immune globulin heavy chain gene was shown on bone marrow aspirate too. The patient was treated with a cyclic therapy including Fludarabine and Cyclophosphamide every 4 weeks for six cycles. By now he is in sufficient general condition.

**PUB39**

HAIRY CELL LEUKEMIA (HCL): RETROSPECTIVE ANALYSIS OF THE EXPERIENCE OF A DIVISION OF INTERNAL MEDICINE

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From April 1995 to June 2000, 10 patients were diagnosed and treated for typical HCL at the Second Division of Internal Medicine of Azienda Ospedaliera S. Maria degli Angeli Pordenone. The patients were admitted at the Division for occasionally found pancitopenia (n=8), intestinal bleeding (n=1), splenomegaly (n=1). Eight were male and 2 female, median age was 56.7 years (range 39–76). A concomitant renal carcinoma was diagnosed in two patients that underwent nephrectomy; one, 75 years old, was put then only under observation, the other was administered chemotherapy (2 - Cda) 2 months later. Diagnosis was performed by bone marrow aspirate - biopsy and bone marrow and peripheral blood immunophenotyping. Six patients were given α–interferon (IFN–α) as first line treatment at a dose of 3MU s.c. every other day for 12 months. In 2 cases was associated with granulocyte colony stimulating factor (G-CSF). No maintenance therapy was administered to responsive patients. One patient developed a severe polineuropatia and discontinued the treatment. Three patients relapsed after 18, 11 and 6 months respectively. One patient was not responder. 2-chlorodeoxyadenosine (2 - Cda) was administered in first time as a 2-hour i.v infusion at the dose of 0.1 mg/kg/ day for 7 days for one course in 3 patients; they all were responsive and are still in complete remission (CR) at 9, 13 and 9 months off therapy. All relapsed/resistant patients following IFN–α, were given 2-Cda at the same dose. Filgrastim 300 ug was given every other day in the presence of neutrophil count lower than 1×10⁹/L. No febrile complications were observed; all cases were responsive and are still in CR 13, 50, 7 and 40 months off therapy. In our experience two points are of relevant importance: first, the high prevalence of the disease (about 2 case over 200.000 people a year), second, the unusual finding of renal carcinoma in two patients. We confirm the high efficacy of 2-Cda in treatment of HCL both as first and as second line treatment. The safety of the therapy, especially if associated with filgrastim allows to treat the HCL patients also in not specialized structures.

**PUB40**

PRIMARY BONE LYMPHOMA. A CASE-REPORT

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Primary lymphoma of bone (PBL) represents an uncommon tumor. It constitutes approximately 5% of all extranodal non-Hodgkin’s lymphoma (NHL). A precise histologic diagnosis is often difficult for the poor quality of the biptic specimens. In a retrospective analysis of 60 cases collected between 1943 and
1996, all the 33 that could be immunophenotyped were of B cell origin, in 92% the diagnosis was of large cell lymphoma, according to the REAL classification. PBL presents most often in the long bones and with Ann Arbor stage I. These statements could be valid only in Western countries, because a different histologic and clinical pattern is described in Chinese patients. Our patient is a 22 years old woman of Albanian origin, HIV negative. Two years before she complained of right headache. At CT scan of the head an osteolytic area of 2-3 cm. in diameter was observed in the right parietal bone, that underwent en bloc resection. The histologic diagnosis was: Langerhans cell histiocytosis. The patient remained symptom-free without any therapy for two years. Then she complained of back pain which irradiated to the left leg. CT scan of the lumbar vertebrae disclosed a derangement of bone structure involving the whole L4 vertebral body. This alteration was confirmed at MRI of the spine. There wasn't spinal cord compression and none of the other vertebrae was involved. No other sign of bone lesions was evident at whole body bone scan with technetium-99m diphosphonate. A vertebral biopsy was performed and the histologic diagnosis was of B cell centrofolicular lymphoma, grade three, or possibly diffuse large cell lymphoma. The growth fraction was quite high (Ki67/MIB-1 33%). The lymphoma could be considered on a practical point of view at high grade of malignancy. Bone marrow biopsy was normal. CT of the thorax and abdomen and Gallium 67 whole body scan did not reveal any other localization. LDH levels were within normal values. We programmed three courses of CHOP, then a restaging to decide further courses of chemotherapy and/or locoregional radiotherapy. After one course the pain has improved and the patient, who was nearly confined to bed, can now stand and walk. Primary NHL of bone has a better prognosis than other extranodal NHL, especially in young patients, the 5-year overall survival being 61%. It can be considered a localised disease, because bone marrow is not usually diffusely infiltrated. Advanced stages are nearly exclusively caused by the presence of multiple bone lesions. In our patient there was a first osteolytic lesion, very probably of lymphomatous nature, which was misdiagnosed and treated with surgery alone. After two years another circumscribed bone lesion appeared at distance from the former, in absence of any other sign of diffusion. Standard therapy are chemotherapy and radiotherapy. In our opinion monoclonal anti-CD20 antibodies (Rituximab) could also have a role in these B-cell lymphomas with low diffusive potential, in order to eradicate minimal residual disease and to prevent late relapses.

**PUB41**

**PRIMARY FOLLICULAR NON-HODGKIN’S LYMPHOMA OF THE LACRIMAL SAC: A CASE REPORT**

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Primary non-Hodgkin’s lymphoma of the lacrimal sac is a rare entity, with most reported cases representing secondary involvement of systemic lymphoproliferative disease. The clinical case of a 50-year old man whom we observed after removal of mass involving the right lacrimal sac area is described. The histological picture of removed tissue showed soft nodular pattern, formed by lymphoblastoid cells and by cleaved cells. Lymphomatous cells showed high positivity for CD20, weak positivity for CD10, negativity for CD5; in spite of negativity for bcl2, immunophenotypic feature (CD20 **+, CD10 −−, CD5 −−) associated with cytomegalovirus-like picture and with nodular pattern of process was indicative for his follicular nature, although fairly atypical. The following staging (body computed tomography, bone marrow biopsy) excluded further sites of lymphoproliferative disease: nevertheless, in consideration of atypical features, the patient, initially, was treated with four cycles of chemotherapy with cyclofosfamide, doxorubicin, vincristine, prednisone, and, next, with regional irradiation. At present, the patient remained free of lymphoma four months after the end of treatment. Features of high malignancy suggest, in localized lymphoma of the lacrimal sac, necessity of associated treatment with chemotherapy and with radiotherapy, as in described case.
The end of chemotherapy.

Is programmed a locally radiotherapy after methotrexate achieving partial remission after only two cycles protocol in accord with GISL program plus CNS prophylaxis with Ann Arbor system. We applied the chemotherapy with BACOP protocol. L., Emilia

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Intestinal T cell lymphoma is a subset of extranodal non Hodgkin's lymphoma and can be associated to adult celiac disease. Enteropathy associated T cell lymphoma is a unique form of non Hodgkin's lymphoma involving the gastrointestinal tract. This disorder is rare and strikes between the ages of 60-70 both male and female with the same frequency. Case report. This 53-years old patient suffered of autoimmune hepatitis (B and C virus: negative) treated from 1991 with a low dosage of steroids. The clinical history began in september 2000. The patient complained of abdominal pain and digestive difficulty with subsequent onset of nausea, anorexia, vomiting and (10-15 kg) weight loss associated with diarrhea. Abdominal ultrasound revealed extension of the small bowel with thickening of the intestinal wall. Gastroscopy revealed gastric stigmata. The study of intestinal transit showed proximal jejunal anse extension with stops of the transit. The patient underwent a partial jejunal resection (cm 17). The histological report was: ulcerative jejunitis associated with villous atrophy type coeliac disease. The ulcer contained T lymphocyte infiltration. Clinical tests revealed antibodies for antigliadin (IgA and IgG), antietiodysmy (IgA) and anti tissue transglutaminase (IgA) positive. Chest-abdominal Computerized Axial Tomography (CAT) and bone marrow biopsy were negative. Discussion. Enteropathy associated T cell lymphoma complicates, in 7% of the cases, celiac disease racing from 3 to 20 years. It has been demonstrated, by recent studies, that a strict gluten-free diet reduces or erases risks of developing tumours like lymphoma. Hence, it is important early reacognition of subclinical forms. In the last years new cases of autoimmune pathology, such hepati- tis, associated to coeliac disease, are increased. The clinical case described is a patient affected by hepatitis for about 10 years. Is it possible to have the coeliac disease at the same time of hepatitis? Conclusions. Prognosis of the enteropathy-associated T cell lymphoma is unfavorable. If lymphoma is located in the small bowel, surgical resection may be followed by years of remis- sion. The role of chemotherapy, either as induction or an high dose in relapse, is still unclear. At the moment, guidelines for salvage therapy of celiac disease at the same time as the hepatitis are unavailable.

Extra nodal non-Hodgkin's lymphoma are rare constituting about 20% of all observed cases. Localizations to the oral cavity represented a varying percentage from 1 to 2%, while those to the soft oral tissues, that generally involve the gingival mucous constituted isolated cases also in the more recent literature. The evidence of these cases is more common in the subjects with immunodeficiency acquired or congenital. In November 99 we recognized a male patient 44 years old with the suspect of gingival NHL. Some months before he noted paresthesia to the lower half lip non responder to the common therapy agents. He was submitted to dental investigation and local biopsy. In the suspect of lymphoma's location he was sent to us for investigation. The clinical findings and the laboratory data were in the norm. The histological revision of the biopsy previously prepared, using also immunohistochimical studies concluded: diffuse large B-cell lymphoma, according to the REAL classification. The staging for images and the bone marrow biopsy excluded other possible foci of disease. The definitive diagnosis was therefore primary NHL of the gingiva, stage IA. The patient was treated with polychemotherapy according to MNCOP-B protocol. Therapy was well tolerated, the hematological and not toxicity has been contained. It achieved rapid control of the initial manifestation of disease. Complete remission has been maintained up to present with a follow up of 14 months. The case described summarizes the main characteristics of this rather uncommon pathology: the difficulty to correct diagnosis at presentation, because the clinical symptoms (paresthesia, gingival painful swelling, necrotizing gingivitis, abnormal mobility of one or more dental elements) mimicked other pathologies of the oral cavity and the importance of the local biopsy. Routine treatment guidelines do not exist because there are only individual cases in literature. Nevertheless the aggressive polychemotherapy represents, in our opinion, the golden standard for treatment escaping sooner or late disseminations of disease, almost always recorded with the use of the radiotherapy as single mode of treatment, also in consideration of the poor prognosis of all extranodal disease.
markers β-hCG and α-fetoprotein. At the diagnosis only one patient demonstrated anemia (Hb 7 g/dL), reticulocytopenia and at the bone marrow sample an almost complete absence of erythroblast. This case has been interpreted as an acquired pure red cell aplasia. The treatment was administered for six cycles in four patients and only for three cycles in one patient because of a progressive course of disease. We observed a 50% response rate (two complete remissions, two partial remissions). Patients in partial remission were treated also with radiotherapy. The median survival from diagnosis is 26 months. Only one patient died for progression of disease. In conclusion, thymoma is a rare neoplasm with interesting therapeutic potential for the hematologist. Only during the follow-up it will be possible to confirm whether thymoma has been cleared and the radiotherapy role of avoiding a possible relapse in the subset of patients in partial remission.

**PUB46**

LIMITED USEFULNESS OF ENDOSCOPIC ULTRASONOGRAPHY IN INITIAL STAGING OF GASTRIC LYMPHOMAS

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In the period 1994-2000, 19 patients affected by gastric non-Hodgkin’s lymphoma (NHL) who were staged by endoscopic ultrasonography (EUS) at diagnosis were seen at our Institution. Six out of 19 patients were affected by high-grade (HG) NHL and 13/19 by MALT NHL. In all 6 HG NHL the EUS did not change the therapeutic approach, since all of them were treated by standard e.v. polichemotherapy (CT) regimens. In this group of patients EUS has been demonstrated more useful in the restaging after completion of CT, to evaluate the response and in helping to decide how to proceed. In the group of MALT NHL 8/13 patients were treated with anti Helicobacter pylori (HP) eradication therapy: in 3/8 this has been the only treatment; 3/8 patients received conventional CT (2 out of these 3 patients showed HG NHL areas in gastric biopsies); in 2/8 oral chlorambucil (CHL) was added (one patient who did not respond to eradicating therapy and the other with advanced disease but poor performance status (PS) that did not allow e.v. CT). Five patients (3 treated with CT, 2 with CHL) did not receive anti-HP therapy; 3 patients because HP-negative at diagnosis, 2 of them because of advanced disease (involved lymphnodes). Only in one of these 13 patients the result of EUS modify the programmed therapy after conventional staging (CT scan, trephine bone marrow biopsy): in fact in this single patient EUS revealed involved perigastric lymph nodes that were not detected by CT scan. In conclusion, EUS has revealed a limited, though present, usefulness at diagnosis in gastric NHL: it remains to be elucidated whether this technique could play a role in restaging of patients after therapies and especially in follow-up for early detection of eventual relapses.

**PUB47**

ASSOCIATION OF AMIFOSTINE AND ERYTHROPOIETIN FOR THE TREATMENT OF MYELODYSPLASTIC SYNDROMES

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Amifostine and rHuEpo may have different mechanisms of action in correcting cytopenias in MDS. Twelve patients (male 6, female) were treated with rHuEpo 150 U/kg/day or every other day for 4-6 weeks in combination with amifostine 200 mg/m² three times per week again for 4 or 6 weeks. Eleven out of 12 were transfusion-dependent and treated previously with a variety of drugs; 7/12 had received rHuEpo as single agent. Anemia was present in 12/12, neutropenia (<1,5×10⁹/L) in 9/12, thrombocytopenia (<100×10⁹/L) in 8/12. Cytogenetic aberrations were present in 7/12 cases; according to IPSS were categorized as follows: 2, Int-2 (FAB: RA), 9, High risk (FAB: 2 RA, 5 RAEB with blast ≤10% and 2 RAEB >10%). One patient was unclassifiable because the karyotype was unavailable. Patients were checked weekly and final results evaluated at day 45. Response to therapy was evaluated as hematologic improvements, according the criteria recently proposed by Cheson et al. Results. Two patients (16.6%) became transfusion independent (complete response), and in 4 more cases (50%) a ≥50% reduction in transfusion support was reached (partial response). Complete neutrophil response (at least 100% increase and absolute increase > 0.5×10⁹/L) was obtained in 3/9 cases (30%) with baseline levels <1.5×10⁹/L, and no partial results were observed. Complete platelet response (absolute increase of at least 30×10⁹/L) was reached in 2/8 (25%) patients with baseline levels <100 × 10⁹/L. The combined therapy was well tolerated; side effects included general malaise (1), fever (2; in 1 case the treatment was stopped), vomiting (1) and skin infection (1). In the evaluation of results it should be noted that 11/12 were high risk according to IPSS (vs 2/12 according to FAB). Moreover other stringent response criteria were adopted compared to published works. In conclusion this combination therapy does not offer substantial advantages in the treatment of cytopenias in MDS compared to data obtained using Amifostine or rHuEpo as single agent.
LEVELS OF REACTIVE OXYGEN SPECIES ARE INCREASED IN MYELODYSPLASTIC SYNDROMES


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Myelodysplastic syndromes (MDS) are clonal disorders characterized by peripheral blood cytopenias and normal or hypercellular dysplastic bone marrow (BM), with different factors of acute leukemia progression, associated to the acquisition of multiple sequential molecular lesions. The discrepancy between peripheral cytopenias and hypercellular bone marrow, has been ascribed to excessive apoptosis, even if the specific pathways underlying this phenomenon have not been completely elucidated. A clue to understanding oxidative stress was given by the hypothesis of a possible genotoxic role mediated by oxidative stress. In order to better understand the possible association of oxidative stress with MDS, we investigated a possible association between oxidative stress by measuring serum levels of reactive oxygen species (ROMs) in the sera of 50 MDS cases. Our MDS panel consisted of: 16 RA, 8 RARS, 18 RAEB, 5 RAEB-t, 2 CMML, hypoplastic MDS 1, M/F 30/20. ROMs serum levels were determined using spectrophotometric methods: d-ROMs test (reference interval 250-300 U.Carr.). Statistical analysis was performed by Wilcoxon’s Test and chi square Test. MDS patients presented higher levels of ROMs with respect to normal controls (Wilcoxon’s Test p=0.00846) and chi square Test (p=0.014), showing that MDS are characterised by higher levels of ROMs, and by a higher frequency of increased ROMs serum levels (chi square Test p=0.017). Furthermore, all the 6 MDS cases demonstrating a complex karyotype presented increased levels of ROMs. No correlation were found between increased ROMs serum levels, FAB subtype and IPSS. Our data show that MDS are characterised by an increase in oxidative stress, as suggested by ROMs serum levels, further substantiating the possible association of oxidative stress and MDS. Furthermore, our observation that all the cases with a complex karyotype demonstrated high ROMs levels, may support the hypothesis of a possible genotoxic role mediated by oxidative damage in MDS.

REFRACTORY ANEMIA AND NON-INSULIN DEPENDENT DIABETES MELLITUS

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The occasional but frequent association between non-insulin dependent diabetes mellitus (NIDDM) and refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS), induced us to investigate this association. From January 1995 to March 2001 we observed in our Division of Hematology 56 patients with primary myelodysplastic syndrome (MDS) (26 RA, 11 RARS, 12 RAEB, 1 RAEB-T, 6 CMML). At diagnosis and before to start any specific or support therapy, we discovered NIDDM in 10/26 patients (38%) with RA and in 5/11 patients (45%) with RARS, total 15 patients of 37 (40.5%) with RA and RARS (mean age 73 years, range 51-91); diagnosis of NIDDM was made previously in other hospitals and already the patients had anti-diabetic therapy. In the patients with other MDS (RAEB, RAEB-T, CMML), we report NIDDM only in 2/19 patients (10.5%). Prevalence of NIDDM in Italy is 3.4% and in Campania 3.7% (ISTAT data 1997). Mean prevalence of NIDDM in Italian adult population (age >40 years) is about 5.5% (II diabete in Italia. Ed. Kurtis, 1996, pp.17-30). Examining age >51 years, both males and females, NIDDM prevalence in Italian provinces is calculated between 2.6% and 18.2% and particularly in Campania (Pozzouli) mean prevalence of NIDDM in adult (age >40 years) is 8.4%, range 3.4%-18.2% according to age (Acta Diabetol 1994; 31:87-90; J Clin Epidemiol 1992; 45:835-9; Diab Nutr Metab 1994; 7:123-9). Therefore these epidemiologic data show, with limitations of few patients, that NIDDM prevalence of 40.5% in patients with RA and RARS is significantly greater versus prevalence national data (5.5%), regional data (8.4%) and data of our patients with same age (51-91 years) affected with hematological and oncological diseases (16%) or other primary MDS (10.5%). Relationship between NIDDM and MDS are not reported in literature examined (Medline database). However there are reports about stimulating effect of insulin-like IGF-I growth factors on hemopoietic progenitors and erythropoietin blood levels (Proc Natl Acad Sci USA 1988; 85:7825-30). We think probable that cytokines and regulator hemopoietic factors equilibrium, influenced by anemia of MDS, anyway causes NIDDM in patients with RA and RARS.

CYCLOSPORIN A IN THE THERAPY OF MYELODYSPLASTIC SYNDROMES

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Sometimes the clinical aspects that consent the diagnosis of myelodysplastic syndrome (MDS) are similar to those that are recognised in aplastic anemia in which autoimmune T cell clones are identified and in which the efficacy of immunosuppressive therapy is described. The cyclosporin A (Cy-A) blocks IL2 production and therefore does not consent the T lymphocyte expansion producing cytokines that limits hemopoietic progenitor proliferative activity. These mechanisms are the same identified in MDS. We report a contribution concerning the efficacy of Cy-A in the therapy of MDS. The main clinical data of the 6 evaluable patients (pt), out of 9 total subjects treated with conventional Cy-A, are reported in the following table:

<table>
<thead>
<tr>
<th>Pts</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>WBC &gt;10³/mm³</th>
<th>Hb g/dL</th>
<th>Plts &gt;10³/mm³</th>
<th>FAB</th>
<th>IPSS</th>
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<td>9.2</td>
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<td>10.1</td>
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<td>7.9</td>
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<td>7.8</td>
<td>7.6</td>
<td>189</td>
<td>RAEB</td>
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</tr>
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<td>7.7</td>
<td>65</td>
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<tr>
<td>A.C.</td>
<td>F</td>
<td>54</td>
<td>1.6</td>
<td>10.4</td>
<td>36</td>
<td>RAEB</td>
<td>Intermediate 1</td>
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</table>

The therapy was useful in two cases (Q.L.: 59 years old, male, RA, 46 XY, IPSS low risk and A.C.: 54 years old, female, 46 XX, IPSS intermediate 1) with improvement of the disease and progressive Hgb increase. After Cy-A the first pt showed Hgb values always > 10 g/dL over 17 month follow up. Nevertheless recently he developed an acute myeloid leukemia. The other pt has reached till now a follow up of 5 months. This subject maintains Hgb values >10 g/dL and a normal life quality. The 3rd (P.A., 50 years old, male, involvement of chromosome 5 and 7, marrow blasts 12%, IPSS: high risk) obtained only a partial response with increase of Hgb and reduction of transfusion requirements. After 2 month therapy Cy-A was stopped and an allogeneic BMT was performed. Now the patient is alive and well. In the other 3 patients (2 males and 1 female) no response was documented and Cy-A administration was interrupted after 1 month. In conclusion Cy-A was useful in 3 out of 6 patients. In all of them the Cy-A administration was well tolerated, without the appearance of side effects. The observed progression in acute leukemia was not ascribed to the immunosuppressive therapy.
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