European Hematology Association (EHA)

EHA is a scientific society aiming to support research, education and clinical practice in hematology. Its main objective is to be useful to scientific researchers, clinicians, medical students, as well as all those working in other fields but who are interested in hematology.

The European Hematology Association was founded in June 1992. Today, EHA – with over 2700 active members from 95 countries – is a consolidated organization that pursues a large and growing number of projects and programs.

EHA aims to promote

- Exchange and dissemination of knowledge and scientific information in the field of hematology.
- Education and training in hematology.
- Medical practice in the area of hematology and the position of hematology as medical discipline.
- Scientific research in hematology.
- Exchange of information for all European doctors, scientists and other professionals interested in hematology.
- A unified European training program in hematology in collaboration with European National Societies of Hematology.

In order to achieve these goals, EHA

- Maintains regular contacts and organizes meetings with all European National Societies of Hematology.
- Holds an annual scientific and educational congress in a major European city; European Cooperative Groups and Networks are encouraged to take advantage of this major event to gather.
- Disseminates medical research, both basic and clinic, through the new journal Haematologica/ The Hematology Journal.
- Has established a link with European National Societies of Hematology and other organizations such as the European Group for Bone Marrow Transplantation, European Association for Hematopathology, European Society of Medical Oncology, and American Society of Hematology.
- Provides postgraduate education through the annual congresses, seminars, courses, workshops and meetings organized in collaboration with the European School of Haematology.
- Has a Fellowship/Grants Program to promote research in hematology.
- Accredits scientific meetings and provides CME accounts in collaboration with the European National Societies for hematology.

If you recognize the need for a strong European Hematology Association and would like to take advantage of the various activities of the Association, you may wish to become a member of the EHA and contribute to its objectives.

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Manuscripts must be written in English and should be prepared according to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, N Engl J Med 1997; 336:309-15 (available from http://www.icmje.org). The first page of the manuscript must contain: (a) title, name and surname of the authors; (b) names of the institution(s) where the research was carried out; (c) a running title of no more than 50 letters; (d) acknowledgments; (e) the name and full postal address of the author to whom correspondence regarding the manuscript as well as requests for abstracts should be sent; (f) three to five key words. To accelerate communication, phone, fax number and e-mail address of the corresponding author should also be included.

Editorials and Perspectives should be concise and should not exceed 4 printed pages. No particular format is required for these articles.

Original Articles should normally be divided into an abstract, introduction, design and methods, results, discussion and references. The abstract must not exceed 250 words and must be structured as follows: background and objective, design and methods, results, interpretation and conclusions. A maximum of 6 relevant tables and/or figures (in total) are allowed. Original articles should not exceed 8 printed pages.

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Letters to the Editor should be no longer than 750 words (a word count should be included by the Authors), can include one or two figures or tables, and should not contain more than ten strictly relevant references. Letters should be signed by no more than six authors and should not exceed 2 printed pages.

Review articles are welcome provided that they carry new information to the reader and not simply a general, dull overview. We favor Decision Making and Problem Solving papers, which may include meta-analyses, guidelines, and recommendations by scientific societies. Updates on molecular basis of disease and on recent advances in molecular biology are very welcome.

Additional papers may be considered for the purely online journal. Because there are no space constraints online, Haematologica on Internet will publish several items deemed by peer review to be scientifically sound and mainly useful as educational papers. These will include case reports, irreplaceable images, educational material from scientific meetings, meeting abstracts, and correspondence reporting comments on articles previously published in the journal.

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Un impegno costante in ematologia.

Al fianco del medico, al servizio del paziente.
ed annually in the January issue of Index Medicus. List all authors when six or fewer; when seven or more, list only the first six and add et al.

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Books and other monographs [personal authors,6 chapter in a book,7 published proceeding paper,8 abstract book,9 monograph in a series,10 agency publication11]:


Forthcoming13 or electronic material14:


References to personal communications and unpublished data should be incorporated in the text and not placed under the numbered References. Please type the references exactly as indicated above and avoid useless punctuation (e.g. periods after the initials of authors’ names or journal abbreviations).

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41° Congress of the Italian Society of Hematology

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Most patients with advanced stage Hodgkin lymphoma can be cured with a standard course of six cycle of ABVD chemotherapy plus involved field (IF) radiotherapy. Patients with less advanced stage or with a more responsive disease could possibly achieve a cure with a shorter course of chemotherapy. In 1992 the GISL addressed the issue of the proper number of chemotherapy cycles planning a response-oriented, ABVD-based trial for intermediate-stage Hodgkin’s lymphoma patients. Patients and Methods. From January 1992 to December 2002, 218 patients younger than 70 were enrolled. Eligible patients had histologically confirmed in 12 cases (6%) and extranodal localizations included lung (6), salivary glands (2), Waldeyer’s ring (2), pleura (1), breast (1), and thyroid (2). Forty-five per cent of cases achieved a CR/CRu within the third ABVD cycle. Overall, at the end of the therapeutic program the results obtained in cytological material of different types were comparable, indicating the high flexibility of the method. However, retrospectively we describe a highly sensitive and specific Western blot (WB) method that allows the easy identification of NPM1 leukemic mutants in cytological samples from AML patients. Rabbit polyclonal antibodies were generated against the altered C-terminal portion of the most common NPM1 mutant protein (type A). WB analysis of a selected number of AML samples proved that these antibodies reacted specifically with the NPM mutant but not with the wild-type protein. These findings prompted us to use this method to analyze systematically cytological leukemic samples and to compare blindly the results obtained by WB with those derived from IHC and, when available, from NPM1 mutational analysis. Totally, 114 AML patients classified by IHC into NPMc+ (n=57) and NPMc- (n=57) were enclosed in the study. WB analysis was performed retrospectively in 82 AML cases and prospectively in 32 cases. We investigated a total of 175 cytological preparations (83 from NPMc+ and 92 from NPMc- AML patients). In NPMc+ AML types, including frozen dry cell pellets of Ficoll-isolated leukemic cells; 1-2 drops of fresh whole blood or peripheral blood; or even cytospins or smears. WB analysis predicted the time of response, early responders treated with 4 ABVD cycles had better 7 year OS (97% vs 83%, p=0.04) and same FFTF (84% vs 83%) compared to late responders, who received 6 ABVD cycles. Conclusions. A response-oriented chemotherapy program is feasible and safe in intermediate-stage Hodgkin’s lymphoma patients. Overall, with a flexible number of ABVD the OS and FFTF of present series matches nicely with that expected for this setting of patients. Early clinical conventional (without PET) restaging identifies cases who have an excellent outcome even with a shortened ABVD course.
**BEST-03**

**MELPHALAN 200 MG/M2 (MEL200) VERSUS MELPHALAN 100 MG/M2 (MEL100) IN NEWLY DIAGNOSED MYELOMA PATIENTS: A PROSPECTIVE, RANDOMIZED PHASE III STUDY**


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**Introduction.** Many studies demonstrated the superiority of melphalan 200 mg/m² (MEL200) and 100 mg/m² (MEL100) to standard therapy. In this randomized, phase III trial, we compared the efficacy and the toxicity of MEL200 with MEL100. The primary end points were CR rate, EFS and incidence of gastrointestinal toxicity, infections and early deaths. **Methods.** Between January 2002 and July 2006, 298 pts were enrolled. Inclusion criteria were previously untreated myeloma, aged < 65 and Durie and Salmon stage II or III. Exclusion criteria were abnormal cardiac, liver and renal function, respiratory disease, HBV, HCV, or HIV positivity, concomitant cancer or psychiatric disease. Induction treatment for both groups: 2 days of dexamethasone-doxorubicin-vincristine debulking courses, 2 cycles of cyclophosphamide plus G-CSF followed by stem cell harvest. Conditioning regimens: 2 cycles of MEL200 or 2 courses of MEL100, followed by stem cell reinfusion. **Results.** At the present, 246 pts (median age 57) were evaluable. 124 pts were randomized to MEL200 and 122 to MEL100. Patient characteristics were similar in both groups. 46 pts did not complete MEL200; 36 did not complete MEL100. CR rate was not different in two arms (17% in MEL200 versus 10% in MEL100, p = 0.2) while the VGPR rate of MEL200 was superior to MEL100 (38% versus 22%, p = 0.011). PR was 80% in MEL200 and 71% in MEL100 pts (p = 0.079). The median follow-up for censored pts was 26.5 months. The 3 years EFS was 48% in the MEL200 arm and 31% in the MEL100 arm (p = 0.01). The 3 years overall survival was 96% in the MEL200 group and 71% in the MEL100 group (p = 0.51). Grade 3-4 hematologic toxicity was comparable in two arms, but 84% of MEL200 pts received >4×10^11 CD34+ cells compared with 52% of MEL100 group (p = 0.001). There was a tendency for more grade 3-4 non-hematologic adverse events after MEL200 (52% versus 34%, p = 0.01 in the 1st cycle and 39% versus 31%, p = 0.9) in the 2nd cycle. The incidence of severe gastrointestinal toxicity, including mucositis, was 51% after MEL200 and 21% after MEL100 (p < 0.001). The incidence of grade 3-4 infections and of early deaths were similar in both group: 54% of MEL200 versus 45% of MEL100 pts (p = 0.25) and 6% in the MEL200 and 4% in the MEL100 arm (p = 0.9), respectively. **Discussion.** MEL200 resulted in a significantly higher VGPR rate but this did not translate in a superior EFS, and it was associated with less gastrointestinal toxicity, including mucositis. The analysis on all pts is planned for July 2007. The updated data will be presented at the meeting.

**BEST-04**

**ECULIZUMAB IMPROVES ANEMIA AND QOL IN PAROXYSMAL NOCTURNAL HEMOLGLOBINURIA: FINAL REPORT OF THE RANDOMIZED PLACEBO-CONTROLLED TRIUMPH STUDY**


PNH is an acquired hemolytic anemia in which RBCs lacking complement inhibitory proteins are sensitive to complement-mediated hemolysis. Intravascular hemolysis in these patients often requires transfusion support and may result in serious morbidities leading to a poor quality of life (QoL), including fatigue, reduced global health status, patient functioning, pain and dyspnea. Eculizumab (EC) is a new terminal complement inhibitor tested in a placebo-controlled randomized phase III trial (TRIUMPH); here is a detailed analysis of the effect of EC on various parameters of anemia, as well as on levels of fatigue and other patient reported outcomes. EC-treated patients, as compared to placebo, showed an 85.8% decrease in intravascular hemolysis (by LDH AUC, p = 0.01) and this reduction in mean transfusion requirement resulted in a 2.5-fold increase in PNH RBC mass (p < 0.001), while no change was observed in placebo-receiving patients; this increase in PNH RBC mass was associated with an increase in hemoglobin levels (p < 0.001). The number of PRBC units transfused decreased from a median of 10.0 with placebo to 0.0 with EC (p < 0.001), regardless of transfusion requirements prior to treatment; 51.2% of EC-treated patients became transfusion independent (versus 0.0% in the placebo arm, p < 0.001). Even patients who required transfusions while on EC showed a marked reduction in transfusion requirement. EC treatment, as compared to placebo, was associated with a dramatic improvement in fatigue as measured by both the FACIT- fatigue and the EORTC QLQ-C30 fatigue scales (standardized effect sizes [SES] = 1.13, p = 0.001 and SES = 1.12, p = 0.001). EC treatment was also associated with significant improvement with moderate to large SES in the following EORTC QLQ-C30 scales: role, global health status (0.57, p = 0.001), social functioning (0.57, p = 0.003), cognitive functioning (0.78, p = 0.002), physical functioning (1.01, p < 0.001), emotional functioning (0.51, p = 0.008), pain (0.65, p = 0.002), dyspnea (0.69, p < 0.001), and appetite loss (0.50, p < 0.001). These data demonstrate that effective control of intravascular hemolysis in PNH by EC results in a substantial improvement of anemia, as evidenced by increase in endogenous RBC mass, improvement of hemoglobin levels, and reduction of transfusion requirement. These results translate into clinically meaningful improvement in patient reported outcomes including fatigue, global health status, patient functioning, and disease-related symptoms in PNH.

**BEST-05**

**OUTCOME OF ALLOGENIC STEM CELL TRANSPLANTATION IN PATIENTS WITH IDIOPATHIC MYELOFIBROSIS: THE G.I.T.M.O. EXPERIENCE**


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**Introduction.** Idiopathic Myelofibrosis (IM) can be cured by allogeneic haematoopoietic stem cell transplantation (HSCT), but HSCT is associated with significant morbility and mortality, making the choice and the timing of transplantation still a matter of debate. **Methods.** We analyzed the data of the Gruppo Italiano Trapianto Midollo Osseo (G.I.T.M.O.) Registry regarding a total of 100 patients with IM, who underwent allogeneic HSCT in 25 different Italian Transplant Centres between 1986 and 2005. Cox proportional hazard models were used to assess the association of various prognostic factors with TRM, EFS and OS. **Results.** Median time between diagnosis and HSCT was 13 months (1-252). At the time of HSCT, median age was 49 years (21-68), 90% of the patients had a Dupriez score ≥1,52% had previously received red
cells transfusions, 24% had blasts in the peripheral blood, 23% had an abnormal karyotype, 56% had splenomegaly, 42% underwent splenectomy before HSCT. Sixty-five transplants were performed between 2001 and 2005. Conditioning regimen was myeloablative in 53% of the cases and at reduced intensity in the remaining 47%. It was based on busulfan plus cyclophosphamide (cy) or fludarabine in 38%, thiopeta plus cy in other 38%, on TBI in 6% and on other drugs associations in 18% of patients. Stem cells came from matched sibling donors for 78% of the patients, mismatched sibling for 4% and unrelated donors for the remaining 18%. Forty-nine per cent received BM cells. Eighty-five per cent achieved full engraftment. One-year TRM was 34%. The estimated 3-year and 5-year survival post transplant were respectively 42% and 31%. Factors associated with an higher TRM rate were: an older transplant date (1996-2000 vs before 1995: HR=0.19 CI95%[0.06;0.66], after 2001 vs before 1995: HR=0.26 CI95%[0.10;0.69], a longer time between diagnosis and HSCT (HR=1.01 CI95%[1.00;1.02]), a myeloablative conditioning regimen based on busulfan and cy (HR=0.25 CI95%[0.10;0.64]), an unrelated donor (HR=2.56 CI95%[1.28;5.09]). A conditioning regimen based on thiotepa plus cy and a sustained engraftment were associated with a longer OS rate (HR=0.44 CI95%[0.23;0.83] and HR=0.38 CI95%[0.17;0.84] respectively). Conclusions. This retrospective large registry study suggests that HSCT should be taken into consideration in patients with MI, particularly those who have sibling donors, early in the course of the disease and that a conditioning regimen with thiopeta and cy should be preferred.

BEST-06
IDENTIFICATION OF DIFFERENTIALLY EXPRESSED miRNA IN GRANULOCYTES FROM PRIMARY MYELOFIBROSIS

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Introduction. Primary Myelofibrosis (PMF) is a clonal disorder of the hematopoietic multipotent stem cell. The recent description of acquired JAK2 V617F and the MPL W515L mutations may represent the first reliable biological molecular markers of the disease even if they are not specific for PMF. aberrantly regulated genes in PMF are still poorly understood, but microRNA might play a role in deregulated gene expression. With the aim to identify possible abnormalities in miRNA regulation in PMF we have performed a comparative analysis of miRNA expression profile of normal and PMF granulocytes. Methods. Using stem loop-primed reverse transcription and TaqMan quantitative real-time PCR (QRT-PCR), the expression of 156 mature miRNAs was evaluated using pooled granulocytes from PMF patients either JAK2V617F wild type or homozygous (two pools each) and control subjects. Twelve differentially expressed miRNA were than validated on additional normal and PMF samples, and their expression was analyzed also in PV and ET granulocytes. Results. There was a global down-regulation of miRNA levels in PMF granulocytes, and 60 out of 125 detectable miRNAs displayed differential expression versus normal samples. Twelve miRNAs, selected for their statistically significant difference, were finally validated. miR-31, -150, and -95 level was significantly lower, while that of miR-190 significantly higher in PMF granulocytes compared not only to control but also to PV or ET samples. However, miR-34a, -342, -326, -105, -149 and -147 which were all reduced compared to controls did not differentiate PMF from PV or ET. Increased levels of miR-182 and -183 correlated with JAK2V617F in homozgyous status. Three putative genes targets (HEBEGH, HMGA2 and MYB), which have been found deregulated in PMF, correlated with expression levels of regulatory miRNA. Discussion. A defined miRNA profile distinguishes PMF from normal granulocytes, and partially also from PV or ET granulocytes.

BEST-07
IMATINIB 400 MG IN LOW SOKAL RISK EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS. RESULTS OF AN OBSERVATIONAL, MULTICENTRIC PROSPECTIVE TRIAL OF THE GIMEMA CML WORKING PARTY

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In controlled studies, chronic myeloid leukemia (CML) patients at low Sokal risk showed a more favourable outcome compared to high and intermediate risk pts. The IRIS study report an overall rate of complete cytogenetic response (CCgR) of 76% and of 89% at 12 and 60 months, respectively (versus 49% and 69% in high risk pts). Nonetheless, to date no prospective study outside strictly monitored frameworks has specifically investigated the role of imatinib (IM) in the treatment of these pts. Methods. Pts were monitored for conventional cytogenetic and molecular response every 3-6 months. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR, Bcr-Abl/Abl x 100 - Taqman) were centralized in Bologna. Major molecular response (MMoR) was defined as BCR-ABL/ABL% less than 0.1. Results. Between January 2004 and December 2005, 202 ECP CML pts at low Sokal risk were enrolled in a study of the GIMEMA WORKING PARTY ON CML (serial n. CML/023) and treated with imatinib (IM) 400 mg daily. 129 (64%) were male and 73 female. The median age at diagnosis was 43 years (range: 19-69). Median observation time is 22 mos (range: 11-44). A complete hematologic response was achieved in all but 1 pt. 179 (89%) pts obtained a CCgR in 6 to 24 mos (median: 6), 156 (77%) within 12 mos. No pt lost the CCgR and 10 pts obtained a partial cytogenetic response, for an overall rate of major CCgR of 94% at 24 mos. 7 pts never achieved a CCgR, after 12 to 24 mos from start of IM (median: 18). 175 of the 179 pts with stable CCgR were evaluable for molecular response. The frequency of MMoR was 30%, 47%, 58% and 71% at 6, 12, 18 and 24 months, whereas the rate of undetectable BCR-ABL levels increased from 1.7% at 6 months to 9% at 24 mos. 132 pts (75%) achieved a MMoR, which was is a failure of RT-Q-PCR negative at all observations. 3 pts discontinued IM, because of progression to accelerated phase (1), allogeneic transplant (1) and persistant hematologic adverse event (1). 2 pts progressed to accelerated phase, after 6 and 13 months of therapy and 3 pts died. 3 pts underwent an allogeneic transplant (1 because of disease progression and 2 because of lack of CCgR at 12 months). At 12 and 24 mos, the estimated rates of OS, EFS and DFS were 99.5%, 99%, 96.7% and 96.5% and 94.7%, respectively. Conclusions. In our study, low Sokal risk pts showed a high rate of CCgR, with early and durable molecular responses, reinforcing the results of the IRIS trial.
Superiority of Double vs Single Autologous Peripheral-blood Stem-cell Transplantation in Multiple Myeloma: Final Analysis of Bologna 96 Study


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The Bologna 96 clinical trial was a phase III study aimed at comparing a single vs double autologous peripheral-blood stem-cell transplantation (ASCT) as up-front therapy for younger patients with symptomatic multiple myeloma (MM). Primary study end point was the complete response (CR) and near CR (nCR) rate. A total of 821 patients were randomly assigned to receive either a single course of melphalan at 200 mg/m² (MEL-200) (arm A: 163 patients) or MEL-200 followed, after 3 to 6 months, by melphalan at 120 mg/m² and busulfan at 12 mg/kg (arm B: 158 patients). In comparison with the control group, random assignment to double ASCT led to significantly improved CR or nCR rate (33% vs 47%, respectively; p=0.008), prolonged relapse-free survival (RFS) of 18 months (median: 24 vs 42 months, respectively; p=0.001) and significantly extended event-free survival (EFS) (median: 23 vs 35 months, respectively; p=0.001). After a median follow-up for survivors of 70 months, the 7-year probability of overall survival (OS) was 46% in arm A and 43% in arm B (p=0.90). In a multivariate analysis of all patients, variables significantly extending RFS and EFS were CR or nCR rate (p=0.001 for both) and random assignment to double ASCT (p=0.001 and =0.005, respectively); attainment of CR or nCR positively affected the length of OS (p=0.001). Analysis of patients who failed at least nCR after one transplantation revealed that double ASCT recipients had a significantly longer RFS (median: 46 vs 22 months for the control group; p<0.001) and EFS (median: 42 vs 22 months for the control group; p<0.001) compared with patients assigned to arm A who received a single ASCT. Although these latter patients had a 7-year OS rate of 47% as compared with 60% for double-transplantation recipients, the sample size was not powered to detect a statistically significant difference between the two groups (p=0.10). In conclusion, in comparison with a single ASCT, double ASCT effected superior CR or nCR rate, RFS and EFS. Administration of a second transplantation and of novel agents, including thalidomide and/or bortezomib, for treating sequential relapses in up to 50% of patients randomly assigned to single ASCT, likely contributed to prolong OS in this group, thereby minimizing a potential survival gain offered by double autologous transplantation. Double ASCT actually remains the standard of care for patients with newly diagnosed MM and less than 65 years of age.

Identification of a New Mechanism of Resistance to Imatinib and Dasatinib in Ph+ Chronic Myeloid Leukemia: Final Results of Phase II Gimurell Trial: The Addition of Rituximab to Doze-Dense and High Dose Chemotherapy (HDC) with Autologous Transplantation (ASCT) Improves the Outcome of Untreated Poor-Prognosis Diffuse Large B-cell Lymphoma (DLBCL)


On behalf of the Gimurell Hematology, ASO S. Giovanni Battista, Turin, Italy

Introduction. The efficacy of Rituximab (R) with dose-dense and HDC in young patients with poor-prognosis DLBCL is under investigation. Methods. 94 pts<61 years with de-novo DLBCL, stage III-IV at aa-IPI 3 on High (H) or Low Intermediate (LI) risk were randomized in a phase II trial (study group: January 2001-December 2004). Treatment consisted in: induction for two months with 4-R-MegaCEOP courses (R 375 mg/m² d1, CTX 1200 mg/m² + EPI 110 mg/m² + VCR 1.4 mg/m² d3 and PDN 40 mg/m² d3-7) every 14 days with G-CSF; 2 courses of intensified chemoimmunotherapy R-MAD (Mitoxantrone 8 mg/m² + ARAC 2000 mg/m²/12h + Dexam 8 mg/m² for 3 days and R 375 mg/m² d4 and before PBSCh harvest) followed by ASCT conditioned by BEAM. Results. median age was 47 yrs (19-60); H risk 47%; BM involvement 28%; LDH >normal 79% and extranodal sites>1 35%. Complete Response was 82%, PR 1% and no response 12%; toxic death was 5%. Few severe early toxicities (WHO grade 3-4) were reported and late toxicity was minor, with no secondary malignancies. With a median follow-up of 48 months, 4-yr FFS and 4-yr OS rates were: 73% and 80%. These results were compared to those ones achieved into 41 pts, with the same clinical characteristics, enrolled in a previous phase I-II trial with up-front HDC and ASCT but without R. Treatment in HDC control group was: an induction treatment lasting two months with MACOPB x 8 weekly infusions followed by the same intensified and HDC regimens (MAD x 2 + BEAM and ASCT). Four-yr FFS and OS in control group were: 44% and 54%. To properly evaluate the efficacy of R-HDC therapy, a Cox’s model was performed to adjust the effect of treatment for potential confounders (age, aa-IPI, BM involvement, extranodal sites, B symptoms). In this multivariable model the risk of failure and death was confirmed as significantly reduced in R-HDC group: adjusted hazard ratio (R-HDC vs HDC) was 0.44 (95% CI=0.24-0.81, p=0.01) for FFS and 0.45 (95% CI=0.22-0.90, p=0.02) for OS. A better outcome for patients treated with R-HDC was observed in both IPI groups: aa-IPI 2-yr FFS 80% vs 53%.

Identification of a New Mechanism of Resistance to Imatinib and Dasatinib in Ph+ Acute Lymphoblastic leukemia (ALL): Overexpression of aberrantly spliced oncogenic Ikarois isorforms


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Background. Human leukemia has been shown to be heterogeneous for the pattern of spliced isoforms of Ikarois (Ik), one of the most important transcription factor in lymphoid cell differentiation. Forced expression of short Ik isoforms, which lack the DNA-binding domain, alters the differentiation capacities of haematopoietic progenitors arresting lineage commitment. Aim. We sought to determine if the expression of aberrantly spliced oncogenic Ik isoforms could contribute to the resistance in Ph+ ALL patients. Methods. We studied Ik gene expression in bone marrow and peripheral blood samples from 29 Ph+ ALL adult patients. 5 newly diagnosed, 16 resistant to imatinib and 8 resistant to dasatinib as second-line treatment. Reverse transcription-polymerase chain reaction (RT-PCR) using specific primers for exon 1 and exon 7 and nucleotide sequencing were performed to identify the specific Ik isoforms. Amplification and genomic sequencing of the regions surrounding the predominant splice donor and acceptor sites at the exon-intron splice junctions were performed in search for mutations. BCR-ABL transcript levels were monitored in each patient by real-time quantitative PCR (RQ-PCR). Results. The expression of only the wild-type Ik1, Ik2 DNA binding isoforms was detected in 3 (10%) patients (2 resistant to dasatinib and 1 to imatinib). In the 26/29 (90%) remaining patients the Ik6 isoform lacking the DNA-binding domain was detected and in 16/26 (62%) it was the predominant isoform. Genomic sequence analysis showed the presence of 2 SNPs: rs10251980 [A/G] and rs10268273 [A/G] in 50% and 36% of patients, respectively, and 2 point mutations in the intronic sequences. We identified a SNP affecting the third base of the triplet codon for a proline (CCC or CCA) in the highly conserved bipartite activation region of the exon 7. Bi-allelic expression pattern of the various Ik isoforms implicates that trans-acting factor(s) possibly affecting splice-site recognition are involved in the generation of the non-DNA binding isoforms. Molecular monitoring showed that the dominant negative Ik6 expression correlated with the BCR-ABL transcript levels suggesting that this alteration could depend on the Bcr-Abl activity. Discussion. These results indicate that the Ik6 proto-oncoprotein, a non-DNA binding spliced Ik isoforms which block B-cell differentiation at the pre B-cell stage and contribute to the tyrosine kinase inhibitor resistance. Acknowledgment. Supported by: European LeukemiaNet, COFIN 2008, AIL.
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Introduction. The GITMO-IIL trial evaluated if an intensified treatment with ASCT is better than conventional chemotherapy (both supplemented with Rituximab) in high-risk FL at diagnosis. Methods. Eligibility: required a FL with aaIPI>1 or IIL>2 score and an age of 18-60. Primary endpoint was EFS. The analysis was intention to treat. Secondary endpoints were PFS, DFS, OS, rate and prognostic value of MR. R-HDS and CHOP-R have been already described (Ladetto et al. ASH 2005, Rambaldi et al. Blood 2002). Planned sample size was 240 to detect a 20% absolute increase in the 3-years EFS. However the trial was stopped at 136 pts due to R-HDS EFS superiority at a planned interim analysis.

Results. Age, stage, LDH, bulky disease, B-symptoms ECOG PS, extranodal disease aIPI, III and were similar in the two arms. CRs were 62% with CHOP-R and 85% with R-HDS (p<0.01). At a median follow-up of 39 months EFS and PFS are 83% and 56% for CHOP-R and 62% and 70% for R-HDS. OS is 81% in each arm. 73% of relapsed R-CHOP pts underwent R-HDS. MRs were 44% after CHOP-R and 80% after R-HDS (p<0.001). MR was associated to a better PFS (p<0.001). Of note, 2.5yrs PFS of pts with or without MR was similar in the two arms (MR: 62% with CHOP-R and 80% with R-HDS) (no MR: 41% for CHOP-R and 49% for R-HDS). MR was the strongest independent prognostic factor for PFS, EFS and DFS by multivariate analysis. Discussion. This is the first phase III trial including MR analysis in a high proportion of pts and comparing intensified versus conventional therapy in the Rituximab age. This trial indicates that: a) R-HDS has a better EFS and PFS in truly high-risk FL patients; b) MR is the strongest outcome predictor available in FL; c) the similar outcome in pts achieving or not MR, regardless of treatment received, indicates that the superior performance of R-HDS is due to its superior MR rate.

Karyotype is a major determinant of prognosis in adult AML patients. Nevertheless, when cytogenetics risk classification systems are applied to AML patients, the MRC and SWOG systems include a large number of patients included in the intermediate risk category. This group represents, by definition, a group with intermediate prognosis for which the evaluation of the best treatment strategy is difficult. To this aim, search for further prognosticators, basically at molecular level (FLT3, NPM), is currently ongoing. We demonstrated that flow-cytometry negativity of bone marrow minimal residual disease (MRD) at the post-consolidation check-point (defined as the presence of <3.5x10^−4 bone marrow residual leukemic cells, BMRLC) has a significant prognostic impact in AML. In the present analysis we aimed at demonstrating that the combined analysis of karyotype and MRD may help in identifying patients with different prognosis within intermediate cytogenetics group. To this purpose we analyzed a group of 127 AML cases entered into the EORTC/CIMNE protocols AML10/AML12 (age <61yrs) or AML13/AML15/AML17 (age >61yrs), consisting in intensive induction and consolidation cycles. The clinico-biological variables evaluated in our model included age, FAB, WBC count, MDR1 phenotype, FLT3 mutations and level of post-consolidation BMRLC, assessed by multiparametric flow-cytometry (MFCC). By applying the maximally selected log-rank statistics, the threshold discriminating MRD negative from positive cases was set at 3.5x10^−4 BMRLC, a level that distinguished discrete subgroups of MRD− and MRD+ patients at the post-Cors time-point. According to the SWOG classification (88/127 (69%) patients had an intermediate risk karyotype. This proportion was even increased (102/127, 80%) when the MRD classification was used. When these patients were stratified according to the level of MRD at the end of consolidation therapy, MRD− ones showed a poor prognosis (5-yrs RFS and OS less than 20% for both) as compared to MRD+ patients (5-yrs RFS and OS 67% and 47%, respectively). Thus, the combination of MFCC and cytogenetics identifies 2 distinct groups: 1) including patients with good karyotype and those with both intermediate karyotype and a MRD− status after consolidation. This group showed a 5-yrs RFS of 67% and an OS of 55%; 2) including patients with poor risk karyotype and those with both intermediate risk karyotype and a MRD+ after consolidation. This category showed a 5-yrs RFS and OS less than 20%, respectively. These results suggest that the combined assessment of MRD by MFCC and cytogenetics may be particularly useful in discriminating subgroups with different outcomes among patients with intermediate risk karyotype. This may represent a surrogate for molecular approaches in order to design risk-based therapeutic programs in a group of AML where karyotype does not represent a major prognosticator due to the underlying genetic heterogeneity.

Figure 1. A: EFS R-HDS vs CHOP-R; B: PFS PCR negative vs PCR positive.
LYMPHOMAS I

CO-001

A PHASE II TRIAL OF FM (ORAL FLUDARABINE AND MITOXANTRONE) CHEMOTHERAPY FOLLOWED BY YTTRIUM 90 (Y) IBITUMOMAB TIUXETAN (ZEVALIN\textsuperscript{\textregistered}) FOR PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA (FL) PATIENTS

Tani M on behalf of the Italian Cooperative Study Group on Lymphoma

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The therapeutic approach to follicular lymphoma (FL) is particularly controversial. One of the effective strategies is the combination of fludarabine-containing regimens with anti-CD20 monoclonal antibody. Single-agent radioimmunotherapy activity, in particular \textsuperscript{90}Y-Ibritumomab Tiuxetan ("Y-IT, Zevalin\textsuperscript{\textregistered}"), has been demonstrated in heavily pretreated FL patients. The results of these studies support a further evaluation of \textsuperscript{90}Y-IT in combination with chemotherapy earlier in the time course of FL. We conducted a prospective, multicenter, non-randomized, phase II study to evaluate the efficacy and safety of a new approach combining induction chemotherapy with oral Fludarabine and Mitoxantrone (FM) followed by consolidation with \textsuperscript{90}Y-IT for patients with previously untreated FL. Patient eligibility was represented by: age more than 18, untreated stage II-IV FL grade I-II; WHO performance status 0-2. Patients were treated with standard FM chemotherapy (with Fludarabine per os at a dose of 40 mg/m\textsuperscript{2}/day for 3 consecutive days) every 28 days for 6 cycles. Patients were restaged 4 to 8 weeks after completion of chemotherapy and those achieving at least a partial response were eligible for \textsuperscript{90}Y-IT. All patients received a single dose of \textsuperscript{90}Y-IT 14.8 MBq/kg (0.4 mCi/kg). Twenty patients have been enrolled 61 patients who all completed the induction chemotherapy. 50 out of 61 were eligible for consolidation treatment with \textsuperscript{90}Y-IT. 25 patients were male and 36 female; the median age was 54 years (range 30-72); 6 were stage II, 20 stage III and 36 stage IV. After the FM chemotherapy, overall response rate was 98% including 71% complete remission (CR) and 27% partial remission and 2% progression disease. Time to event analyses, including TTP and duration of response are pending further follow-up. Treatment was well tolerated; grade 3-4 hematologic AEs (mostly neutropenia) were seen in 37 patients. Among the 50 patients subsequently treated with \textsuperscript{90}Y-IT, 30/50 (20%) improved their remission status from PR to CR. \textsuperscript{90}Y-IT toxicity included grade 3-4 hematologic AEs in 36 patients (mostly neutropenia and thrombocytopenia). These preliminary data indicate that radioimmunotherapy (RTI) appears highly effective and feasible as consolidation after chemotherapy, improving quality of response with acceptable haematological toxicity. Data on the ability of front-line treatment with FM followed by \textsuperscript{90}Y-IT to induce molecular response (PCR analysis of bcl-2/IgH rearrangement) have also been analysed.

CO-002

PRETRANSPLANTATION PET AS MAJOR PROGNOSTIC DISCRIMINANT IN IGEV-TREATED PATIENTS WITH REFRACTORY/RELAPSED HODGKIN’S LYMPHOMA (HL)

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In the last 20 years, the major improvement over the use of CHOP has been the addition of anti-CD20 immunotherapy (Rituximab). Recently, the preliminary data of a phase II trial have showed that \textsuperscript{90}Y-Ibritumomab Tiuxetan ("Y-IT, Zevalin\textsuperscript{\textregistered}") have useful activity in the treatment of relapsed/refractory elderly DLBCL (Morschhauser et al., Blood 2004), with no unexpected toxicities observed. The results of this study support a further evaluation of \textsuperscript{90}Y-IT in combination with chemotherapy earlier in the time course of elderly DLBCL. We conducted a prospective, single-arm, non-randomized, phase II to evaluate the efficacy and safety of \textsuperscript{90}Y-IT of a novel new approach combining induction chemotherapy with CHOP followed by consolidation with \textsuperscript{90}Y-IT for patients with previously untreated elderly DLBCL. Patient eligibility was represented by: patients older than 60 years with biopsy proven, untreated, bidimensionally measurable stage II, stage III, or stage IV DLBCL expressing the CD20 antigen; WHO performance status of 0 to 2. Patients were treated with standard CHOP chemotherapy every 21 days for 6 cycles. Patients were restaged 4 to 6 weeks after completion of the 6 cycle of CHOP chemotherapy. Patients achieving at least a partial response after chemotherapy were eligible for consolidation with \textsuperscript{90}Y-IT provided the granulocyte count was greater than 1500/microl the platelet count exceeded 100,000/microl, and the bone marrow examination at the completion of chemotherapy demonstrated no more than 25% involvement with lymphoma. All patients were to receive a single dose of \textsuperscript{90}Y-IT 14.8 MBq/kg (0.4 mCi/kg). Twenty patients have been enrolled: 12 were male and 8 female; the median age was 68 years (range 61-84); 6 were stage II, 14 stage III-IV. After the CHOP treatment the overall response rate was 100%, including 15 (75%) CR and 5 (25%) PR. Treatment was well tolerated; grade 3-4 AEs were seen in 13 patients; the most common grade 3-4 AEs was neutropenia. After the treatment with \textsuperscript{90}Y-IT, 4/5 (80%) patients improved their remission status from PR to CR. \textsuperscript{90}Y-IT toxicity included grade 3-4 hematologic AEs in 11/20 patients; the most common grade 3-4 AEs were neutropenia (11 patients) and thrombocytopenia (7 patients). Time to event analyses, including TTP and duration of response are pending further follow-up. These preliminary data indicate the feasibility, tolerability, and efficacy of the CHOP plus \textsuperscript{90}Y-IT regimen for patients with untreated elderly DLBCL.
CO-004
RITUXIMAB COMBINED WITH M/VACOP-B AND RADIOTHERAPY IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL): A PROSPECTIVE ITALIAN PHASE II STUDY OF INTERGRUPPO ITALIANO LINFOMI (IIL)
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Introduction. Third generation regimens such as MACOP-B or VACOP-B (M/VACOP-B) in combination with involved-field radiotherapy (IFRT) seem to improve lymphoma-free survival of PMLBCL. The superiority of R-CHOP over CHOP-like regimens has been demonstrated in younger low risk patients, the additional benefit of adding Rituximab to CHOP has also improved the survival of PMLBCL. Aims. To evaluate the effectiveness and safety of Rituximab added to the standard M/VACOP-B regimen (R-M/VACOP-B) ± IFRT in PMLBCL. Patients and Methods. At this time, a total of 45 patients with PMLBCL have been in six participating centers between February 2002 and July 2006. The median age was 58 years (range 17-66); 24/21 (53%) were females; 32 patients had stage II and 13 stage IV; 42 (95%) presented a bulky disease; LDH was increased in 31 (69%) and 24/55 (44%) had a superior vena cava syndrome. According to the age-adjusted IPI score, 30 patients had an IPI = 0-1 and 15 an IPI ≥ 2. All patients were treated with standard MACOP-B (35 patients) or VACOP-B (10 patients) regimens plus six cycles of Rituximab (375 mg/m²) given at weeks 5,7,9,11,13. Thirty-one patients (69%) received medistinal IFRT at a median dose of 36 Gy. The response was evaluated in all patients after 6 cycles of chemotherapy at the end of the planned chemotherapy and after IFRT. Results. The response rate after 6 cycles of the planned R-M/VACOP-B regimen was CR/CRu = 20 (44%), PR ≥ 25% and NR = 1 (3%). Six/45 patients received an early intensification therapy followed by HDT-ASCT because considered low responders (less than PR or progressive disease) during M/VACOP-B chemotherapy. At the end of the chemo-immunotherapy program, 25 patients witnessed a CR/CRu (70%) and 17 a PR (50%). Nine/17 PR patients obtained a CR/CRu following IFRT for an overall CR/CRu rate of 82% (37/45). Three patient relapsed at 12, 12 and 19 months from CR and died of progressive disease despite salvage therapy. After a median follow-up of 25 months, the 3-year OS and PFS were 80% and 78%, respectively. No significant differences in terms of PFS and OS were associated with the IPI score. No additional toxicities related to Rituximab infusion were observed. Discussion. R-M/VACOP-B is active in PMLBCL, allowing the avoidance of severe toxicity for the management of patients with PMLBCL. Consolidation radiotherapy seems to improve the quality of response. Further studies are required to demonstrate if the addition of Rituximab to M/VACOP-B regimens might improve the overall rate of response and survival.

CO-005
PROSPECTIVE IELSG/IIL STUDY IN PRIMARY DIFFUSE LARGE B-CELL LYMPHOMA OF THE TESTIS (PTL): IMPROVED OUTCOME WITH RITUXIMAB (R)-CHOP WITH CNS AND CONTRALATERAL TESTIS PROPHYLAXIS
On behalf of International Extranodal Lymphoma Study Group (IELSG) and Intergruppo Italiano Linfomi (IIL), Hematology ASO S. Giovanni Battista, Turin, Italy

Introduction. PTL has a poor prognosis with a 5-yr overall survival of 40-55%. Causes of failures are: contralateral tests, CNS and extranodal relapses. The IELSG 10/IIL study is a prospective phase II international trial for stage I or II PTL aimed at defining a standard treatment for PTL. Methods. Between June 2001 and December 2006, 53 pts with untreated stage I-II PTL were enrolled. Treatment was: R-CHOP21 (R 375 mg/m², Cx 750mg/m², Doxo 50 mg/m², Ver 1.4 mg/m² d 1 and Pdn 40 mg/mq d 1-5) for 6 or 8 (in stage II pts with slow response) courses; intrathecal methotrexate (IT MTX) 15 mg for 4 doses in courses 1 and 2; after chemotherapy 30Gy scrotal RT to the contralateral testes was planned to all pts reserving 30-36Gy on loco-regional nodes for stage II disease. Results. To date 49 pts who have completed the treatment are evaluable. Median age was 65 years (range 22-80); 38 stage I and 11 stage II; 3 had bilateral testicular involvement; 5 LDH > normal and 2 B symptoms. All received R-chemotherapy as planned. Forty-six (92%) pts received adequate CNS prophylaxis (at least 4 IT MTX), 3 less than 4 IT because of poor tolerance or toxicity. Scrotal RT ≥ nodal RT was given to 44 pts (90%); 5 did not perform it (3 refusals, 1 progressive disease and 1 bilateral orchietomy). Forty-eight pts (98%) achieved a CR and 1 progressed after 4 R-CHOP. With a median follow-up of 36 months, 3-yr OS, 3-yr PFS and 3-yr EFS were: 87% (95% CI 69-96%), 84% (95% CI 65-93%) and 79% (95% CI 60-90%) respectively. Seven pts relapsed or progressed: 2 in nodal sites, 3 in extranodal + nodal sites and 2 in CNS (1 isolated meningeal and 1 meningeal + nodal relapse). The actuarial risk of CNS relapse at 3 years is 2.5% (95% CI 0-7%). No contralateral testis relapses were observed. Five patients died: three because of DLBCL, 1 of heart failure and one of acute myeloid leukaemia 21 months off therapy while in CR. No toxic deaths occurred during treatment. Main grade 3-4 toxicities were: hematological 26% and neurological 14%. Conclusions. This is the first prospective study in PTL with R-CHOP and complete CNS and scrotal prophylaxis. Contralateral testis relapses were not observed and the incidence of CNS relapse seems to be reduced. These results compare favourably with those previously reported in the literature. However, if further relapses will be observed after a longer follow-up, there would be a need for innovative strategies to address the issue of systemic and CNS relapses.
**CO-007**

**A SCREENING STUDY OF CHLAMYDIA PSITTACI INFECTION IN 172 CASES OF NODAL AND EXTRANODAL NON-HODGKIN LYMPHOMAS**

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**Background.** Ocular adnexal lymphomas (OAL) are associated with Chlamydia psittaci (Cp) infection. The prevalence of this infection in other extranodal or nodal non-Hodgkin lymphomas remains to be defined.

**Methods.** 172 non-consecutive cases of extra-orbital non-Hodgkin lymphomas were analyzed: 129 were extranodal B-cell lymphomas (73 DLBCL and 56 MZL), 12 were extranodal T-cell lymphomas (6 anaplastic large cell, 6 mycosis fungoides), and 31 were nodal B-cell lymphomas (11 follicular, 10 DLBCL, 10 mantle cell). Thirty-one patients with non-specific tonsillitis, 36 with non-specific reactive lymphadenopathies and 7 non-neoplastic spleens were used as controls (n= 74). After central pathology review, DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks and amplified by a multiplex touchdown PCR assay, as previously reported [Ferrenti AJM, et al. JNCI. 2004]. Results. Cp DNA was detected in 17 (10%) cases of NHL, C. pneumoniae and C. tao-
chonatii DNA was detected in 3 (2%) cases (p<0.001). Cp DNA was present in 5 (7%) out of 74 controls, in 13 (10%) of the 129 cases of extra-
nodal B-cell lymphomas, in 2 (17%) of the 12 extranodal T-cell lymphomas, and in 2 (6%) of the 31 nodal B-cell lymphomas; Cp DNA was detected in 4 (7%) of the 56 MZL cases and in 9 (15%) of the 73 DLB-
CL cases (p=0.33). Cp DNA was not randomly distributed among extranodal B-cell lymphomas: it was detected in 6 (25%) of 24 cases of cuta-
neous lymphoma (p=0.02, in comparison to controls), in 3 (18%) of the 17 lymphomas of the Waldeyer’s ring (p=0.16), and in 4 (8%) of the 53 gastrointestinal lymphomas (p=0.9), while it was absent in B-cell NHL arising in the lungs, salivary glands and spleen. Conclusions. This is the largest study revealing that Cp-related lymphomas may occur outside the oral cavity. Since these lymphomas may arise in the skin and Waldeyer’s ring, two extranodal sites considered as first-barrier to antigen expos-
eur. This finding may have obvious clinical implications, in view of the encouraging results offered by Cp-eradicating therapy with doxy-
cycline in the treatment of OAL. These figures should be considered in view of the possible differences in the prevalence rate of Cp infection
in OAL among different geographical regions reported in the literature.

**Conclusions.** This is the first follow-up report concerning the prevalence of Cp infection in NHL. The prevalence of this infection in oth-
er extranodal or nodal non-Hodgkin lymphomas remains to be defined. According to the data reported by literature, demonstrates that
Cp-positive patients (potential occult carriers) but also in anti-HBc positive patients (potential occult carriers) with non-Hodgkin’s lymphoma undergoing chemo-immunosuppressive treatment particularly associated to Immunotherapy. We systematically screened for HBV markers 270 consecutive non-Hodgkin’s lymphoma patients examined from 1994 to 2007 at Cagliari’s Haematological Unit. Of them 335 (38%) were found to be potential occult carriers. Of them 250 (75%) were treated. Of these 250 anti-HBc-positive patients 101 (40%) had rituxi-
mar included in the regimen. The other 149 patients (59%) were treated with standard chemotherapy. None received lamivudine. Globally 4
of the 335 anti-HBc-positive patients (1.2%) experienced acute hepatitis
associated with the re-emergence of HBsAg (reverse seroconversion). Two of them have been treated with rituximab associated with cytotox-
ic therapy (2 out of 101 =2%). The other two have been treated with CHOP-like regimen (2 out of 149 =1.3%). There was no significant dif-
ferece between the two groups of patients (treated or not with rituxi-
mar) (p=0.693). In one case the HBV-reactivation occurred during immuno-chemotherapy (that was suspended and never continued). In the other three cases the reactivation occurred from five to seven months after the end of cytotoxic therapy. All the four patients clinically recov-
ered from the HBV reactivation with long-term persistence of HBsAg. Two patients were treated with lamivudine (unavailable for the other
two patients). The outcome of the two treated patients was clinical and virological remission. Both obtained NHL complete remission. One patient, who didn’t complete chemotherapy because of HBV reactiva-
tion, later experienced NHL relapse and was treated, under adefovir pro-
phylaxis, with a salvage therapy and achieved a second complete remis-
sion. The two untreated patients died as a consequence of the progres-
sion lymphoma even if they had completed chemotherapy. Our expe-
rience, according to the data reported by literature, demonstrates that
HBsAg-negative/anticore-positive patients are at low risk for HBV reac-
tivation during and after chemotherapy and suggests the need of identi-
fying a correct strategy for the treatment of these patients.

**CO-008**

**PRE-EMPTIVE TREATMENT WITH NUCLEOSIDE ANALOGS IN HBV OCCULT CARRIER PATIENTS WITH NON-HODGKIN LYMPHOMA: AN OPEN QUESTION**

Targhetta C,1 Cabras MG,1 Mamusa AM,1 Derudas D,1 Sanna M,2 Angelucci E1

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The risk of Hepatitis B Virus (HBV) reactivation is a frequent and potentially fatal complication in HBV-overt carriers with non-Hodgkin’s lymphoma receiving chemo-immunosuppressive therapy. Numerous studies have demonstrated that the antiviral prophylaxis with lamivu-
dine significantly decreases the incidence of HBV reactivation. Thus the current practice guidelines recommend the pre-emptive administration of lamivudine before initiating chemo-immunosuppressive treatment in HBsAg-positive patients with non-Hodgkin’s lymphoma. The recent introduction of monoclonal antibodies such as rituximab (anti-CD20), used alone or associated with the traditional cytotoxic chemotherapy, has been reported to induce HBV reactivation not only in HBsAg-positive patients (HBV-overt carriers) but also in anti-HBc positive/anti-HBs-negative patients (potential occult carriers), leading in a few instances to severe hepatic complication and even HBV related death. However no systematic study has been reported and evidence is anecdotal. These reports have posed the question whether it’s necessary to administrate the antiviral prophylaxis with lamivudine or not in HBsAg-negative/anti-
HBc-positive patients (potential occult carriers) with non-Hodgkin’s lymphoma undergoing chemo-immunosuppressive treatment particularly

**CO-008**

**PRE-EMPTIVE TREATMENT WITH NUCLEOSIDE ANALOGS IN HBV OCCULT CARRIER PATIENTS WITH NON-HODGKIN LYMPHOMA: AN OPEN QUESTION**

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HBc-positive patients (potential occult carriers) with non-Hodgkin’s lymphoma undergoing chemo-immunosuppressive treatment particularly
ACUTE LEUKEMIAS I

CO-009
PHENOTYPIC AND MOLECULAR FEATURES OF ACUTE PROMYELOCYTIC LEUKEMIA PATIENTS DEVELOPING RETINOIC ACID SYNDROME


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Although all-trans retinoic acid (ATRA) is usually well tolerated for the treatment of newly diagnosed APL, a sizable portion of patients are known to develop the so-called retinoic acid syndrome (RAS) during induction therapy. We analysed here the biologic and clinical characteristics of 110 consecutive patients with genetically proven APL treated with AIDA protocol at a single Institution, with the aim of identifying predictive features of developing the syndrome. The diagnosis of a definitely present RAS (according to Frankel Ann Int Med 1994) was clinically established by the presence of at least three of the following signs: weight gain, respiratory distress, unexplained fever, interstitial pulmonary infiltrates, pleural or pericardial effusions, hypotension, renal failure. A total of 15 patients (13.6%) developed RAS. Median time of interval was 4 days (range 3-5). There were 9 males and 6 females, median age 48 years (range 12-65). According to FAB classification, 5 patients were variant type and according to Sanz risk classification 2 patients were low risk, 6 intermediate and 7 high risk. Median WBC count was 5.0×10⁹/L (range 0.3-50.8) and median platelet count was 27×10⁹/L (range 13-127). Molecular analysis showed the presence of bcr1 in 5 (53%) and bcr2 in 10 patients (67%). FLT3-ITD analysis revealed the presence of an ITD mutation in 8/15 (54%) tested patients. All received desamethasone (10 mg/m²) at the occurrence of symptoms (median 6 days). Respiratory distress was the first sign of RAS in 12/15 patients (80%), of RAS occurred in a median time of 4 days (range 2-8). All the patients had resolution of RAS and reached a complete hematologic remission. Respiratory distress was the first sign of RAS in 12/15 patients (80%), fever was revealed in 12 patients, weight gain in all patients (100%) and renal failure in 2 patients. Pleural/pericardial effusions were revealed in 3 patients and in 4 patients headache was associated to other symptoms. Compared to patients who did not develop the syndrome, patients with RAS had more frequent M3 morphology (50% vs 26%, p=0.02), higher median WBC count (6×10⁹/L vs 2.8×10⁹/L, p=0.01), higher prevalence of the bcr2 isoform (66% vs 44%, p=0.001) and of the FLT3-ITD mutation (53% vs 13%, p=0.002). In addition, as regarding correlation with immunophenotype, a higher incidence of CD2 and CD15 expression was detected in the former group (47% vs 13%, p=0.0001, and 60% vs 0%, p=0.0001). In conclusion, APL patients developing RAS have distinctive biologic characteristics at presentation including consistent immunophenotypic and molecular features.

CO-010
PROGNOSTIC VALUE OF DNA INDEX IN CHILDHOOD ACUTE LYMHOBLASTIC LEUKEMIA TREATED WITH BFM BASED THERAPY: LONG TERM RESULTS OF THE AIEOP-ALL 95 STUDY


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Purpose. Between May 1995 and August 2000 the Associazione Italiana di Ematologia Oncologia Pediatrica (AIEOP) conducted the ALL 95 study for risk-directed, BFM-oriented therapy of childhood acute lymphoblastic leukemia (ALL), aimed at exploring treatment reduction in standard risk (SR), intensification during continuation therapy in intermediate risk (IR) as randomized questions and treatment intensification in high risk (HR). The prognostic value of DNA index was explored in this setting. Patients and Methods. 1,744 patients were enrolled, 115 SR, 1,385 IR, and 244 HR risk. SR patients (DNA index ≥1.16 and <1.60, age 2-5 and WBC<20K, no high risk features), received a reduced induction therapy; IR patients were randomized to receive or not vincristine + dexamethasone (VCR-DEXA) pulses during maintenance; HR therapy was based on a conventional BFM schedule intensified with three chemotherapy blocks followed by double reinduction phase. Results. The event-free-survival and overall survival probabilities at 10 years for the entire group were 72.5% (SE 1.3) and 83.6% (0.9); 85.0% (3.4) and 95.5% (2.0) in SR, 75.1% (1.5) and 87.5% (0.9) in IR, 51.0% (8.2) and 57.2% (3.8) in HR patients, respectively. Patients with favorable DNA index had superior EFS in both IR [83.8(2.7) versus 73.9(1.7)] and in HR [67.8(4.9) and 49.6(3.5)]. Of the 6 patients with DNA Index <0.1 only 1 remained in remission. Conclusions. Favorable DNA index was associated with a better prognosis in IR and HR patients defined by presenting clinical criteria and treated with a BFM-oriented chemotherapy.

CO-011
ABSENCE OF NUCLEOPHOSMIN LEUKEMIC MUTANTS IN B AND T CELLS FROM AML WITH NPM1 MUTATIONS: IMPLICATIONS FOR THE CELL OF ORIGIN OF NPMC+ AML


1Hematology, University of Bari, Bari; 2Hematology, University of Perugia, Perugia; 3Hematology, University of Foggia, Foggia; 4Hematology, University of Catania, Italy

Studies of cell lineage involvement and of cell origin in acute myeloid leukemia with normal karyotype (AML-NK) have been traditionally obstructed by the lack of clonality markers. NPM1 mutations, the most frequent genetic alteration in AML-NK, represent an ideal genetic alteration for tracking cell lineage involvement in AML, as they are usually expressed in all leukemic cells, are very stable and generate mutated proteins which are aberrantly expressed in the cytoplasm. Thus, NPM cytoplasmic positivity (NPMc+) represents a unique tool for studying by immunohistochemistry cell lineage involvement in fixed paraffin-embedded bone marrow samples from NPMc+ AML patients. Combining immunohistochemistry with analysis of NPM1 mutations on laser-microdissected hemopoietic cells, we previously found involvement of two or more myeloid lineages in a large proportion of NPMc+ AML. Lymphoid cells seem to be spared from NPM1 mutations in the three patients studied. However, it can not be excluded that lymphoid cells may be involved in a proportion of NPMc+ AML cases. Thus, the issue on the nature of lymphoid cells in NPMc+ AML is not yet fully clarified. Goals of the present study were to further expand the analysis of lymphoid cell involvement other than NK cells in a large number of NPMc+ AML cases and to definitively clarify whether B and T cells from NPMc+ AML patients belong or not to the leukemic clone. For this purpose, we used anti-NPM antibodies to detect NPM leukemic mutant proteins in lymphoid cells in paraffin-embedded bone marrow biopsies from 20 NPMc+ AML patients. Double-staining for CD20/NPM and CD3/NPM showed a clear nucleus-redirected positivity for NPM in B (CD20+) and T (CD3+) cell lineages. These findings allow the conclusion that in AML-NK cases truly lack expression of NPM mutants, we used double-staining polyonal antibodies that recognize specifically the mutated but not the wild-type NPM proteins. Notably, B and T cells showed complete (nuclear and cytoplasmic) negativity for the NPM mutants. These data were further supported by the results of Western Blot analysis of NPM mutant protein expression on either T (seven cases) or B (two cases) cells purified from peripheral blood of NPMc+ AML patients: none of the cases studied showed positivity for NPM mutant protein. These findings provide strong evidence that either bone marrow or peripheral blood of NPMc+ AML patients do not belong to the leukemic clone. Absence of NPM mutants was also demonstrated in 723 human lymphoid neoplasms of different types. These findings further
support the view that NPM1 mutations are a myeloid-specific genetic alteration and have important implications for the cell of origin of NPM1-AML.

**CO-012**

**MODULATION OF TRYPHTHAN CATABOLISM BY ACUTE MYELOID LEUKEMIA CELLS ACTS AS A GENERAL MECHANISM OF IMMUNE TOLERANCE VIA THE INDUCTION OF T REGULATORY CELLS**


1 Institute of Hematology and Medical Oncology L. & A. Seraglioni, University of Bologna and Stem Cell Center, S. Orsola-Malpighi Hospital, Bologna; 2 Department of Genetics, Biology and Biochemistry, Research Center on Experimental Medicine (CErMS), University of Turin; Turin; 3 Hematology Unit, University of Turin and Hematological Oncology Laboratory, CErMS, Turin; 4 Institute of Hematology and Medical Oncology L. & A. Seraglioni, Hematology and Hematopathology Units, S. Orsola-Malpighi Hospital, University of Bologna, Italy

Indoleamine 2,3-dioxygenase (IDO) enzyme, which catalyzes the conversion of tryptophan to kynurenine, has been identified as a novel immunosuppressive agent by inhibiting T-cell proliferation and is involved in tolerance induction to tumors. We have recently shown that IDO protein is constitutively expressed in a significant subset of newly diagnosed acute myeloid leukemia (AML) patients, resulting in tryptophan catabolism along the kynurenine pathway and in the inhibition of allogeneic T-cell proliferation. Moreover, we demonstrated that IDO-expressing AML cells are capable to promote the differentiation of new CD4+ CD25+ Foxp3+ T regulatory cells (Treg cells). AML cells may be differentiated into leukemic dendritic cells (AML-DCs) which have increased immunogenicity and may be used as vaccine against leukemia. In order to evaluate the immunological potential of IDO+ AML cells, we differentiated the AML cells into leukemic dendritic cells (AML-DCs) from 7 AML samples according to standard procedures and tested IDO expression and activity. At baseline, 5/7 AML samples expressed IDO, whereas 2/7 did not. After differentiation into DCs, IDO+ AML cells showed an up-regulation of IDO mRNA and protein, and IDO+ AML cells turned positive. IDO-expressing AML-DCs were capable to catabolize tryptophan to kynurenine metabolite and, functionally, they inhibited allogeneic T-cell proliferation through an IDO-dependent mechanism. Similarly to undifferentiated IDO+ AML blasts, IDO-expressing AML-DCs induced a population of CD4+ CD25+ Foxp3+ Treg cells, which were capable to suppress in vitro allogeneic naïve T-cell proliferation. These data identify IDO-mediated catabolism as a general tolerogenic mechanism in AML cells, including AML-DCs and raise several concerns for the use of AML-DCs as cellular vaccine against leukemia.

**CO-013**

**THE CYTOSOLIC SEQUESTERATION OF NF-κB INDUCED BY DELOCALIZED NPM1 MAY REPRESENT ONE OF THE MECHANISM RESPONSIBLE FOR THE BETTER CHEMOSENSITIVITY OBSERVED IN NPM1 MUTATED AML PATIENTS**


1 Department of Clinical and Biological Sciences, University of Turin; 2 Department of Haematology, Seraglioni Institute, University of Bologna, Italy

Mutations in NPM1 exon 12 and the resulting shift of NPM1 into the cytoplasm are the most specific and frequent events in acute myeloid leukemia patients with normal karyotype. Cytoplasmatic NPM1 is associated with responsiveness to chemotherapy, although its role in predicting outcome remains to be defined. The aim of the study was to identify the molecular mechanism responsible for chemosensitivity in leukemic cells carrying the mutation of NPM1 (NPM1-m). NF-κB is a transcription factor involved in many intracellular pathways including apoptosis. NF-κB is a heterodimer of p50 and p65 subunits sequestered in the cytoplasm in its inactive form through interaction with inhibitory IκB proteins and activated in the nucleus after degradation of IκB. The activation of NF-κB is responsible for chemoresistance to different drugs including anthracyclines. The aim of the study was to analyze the possible cytoplasmatic interaction between the mutated form of NPM1 and NF-κB. The NF-κB DNA binding activity was analyzed in 72 BM samples collected from AML patients carrying the NPM1 mutations (n=35) and NPM1 wild type (NPM1wt) (n=37) using EMSA and ELISA methods. Immunofluorescence analysis showed Western blot using NPM1 and p65 antibodies were performed to identify the amount and localization of both proteins. Co-immunoprecipitation assay was performed to study the interaction of the two proteins. To study the in vitro chemosensitivity, etoposide incubations were performed in both NPM1wt and NPM1-m cells and apoptosis was evaluated. Downstream genes transcriptionally activated by NF-κB have been evaluated by RT-PCR and RQ-PCR. NPM1wt cells were more sensitive in vitro to etoposide induced apoptosis as compared to NPM1wt (p=0.004). We found a significant lower DNA binding activity in NPM1wt cells when compared to the NPM1-mt cells (p=0.001). Immunofluorescence analysis confirmed the cytoplasmatic localization of NPM1-protein and the prevalent nuclear localization of NPM1wt. In addition, immunofluorescence analysis shows different pattern of NF-κB localization in NPM1-wt cells when compared to NPM1mt cells. In particular, in NPM1wt cells NF-κB is mainly localized in the cytoplasm in the inactive form and in NPM1mt cells is mainly nuclear localized. These data were confirmed by Western blot. The cytosolic interaction of NPM1wt and NF-κB was demonstrated by co-immunoprecipitation studies. Furthermore, the expression levels of genes activated by NF-κB such as bcl2 were significantly deregulated in NPM1mt cells. Finally, TNFα, a potent stimulus for NF-κB activation was unable to activate NF-κB in NPM1mt cells. We demonstrated that p65 and NPM1 interact with each other within the cytoplasm and this interaction results in the sequestration of NF-κB within the cytoplasm. The cytosolic localization of the inactive form of NF-κB explains the reduced NF-κB DNA binding activity observed in NPM1mt patients. These data may provide a possible explanation for the chemosensitivity observed in NPM1mt patients. Furthermore, since NF-κB is involved in the transcription of many genes which regulate proliferation and differentiation processes, the disruption of NF-κB function may represent one of the mechanism of leukemogenesis induced by NPM1 mutated proteins.
**CO-015**

**NUCLEOPHOSMIN MUTATED BLASTS EXPRESS HIGH LEVELS OF SPONTANEOUS APOPTOSIS IN ACUTE MYELOID LEUKAEMIA (AML)**


*Department of Hematology, University For Vergata, Roma, Italy*

Mutated nucleophosmin (NPM1) genes target about 55% of adult AML with normal karyotype and are linked to superior outcome (Falini, 2007). Moreover, mutations and FLT3-ITD are involved in apoptosis and cytokines, which could be relevant for the clinical outcome of patients with AML (Stoyanova et al., 2006). In the present study, we investigated the role of NPM1 mutations in the context of AML with inv(16) (Falini et al., 2007). In the context of AML with inv(16), KIT mutational status appeared not to influence the clinical outcome.

**Introduction.** Several studies have recently pointed out the adverse impact of KIT mutations (mutKIT) on relapse incidence and overall survival (OS) in patients with t(8;21) AML. By contrast, the prognostic significance of mutKIT in patients with inv(16) is still inconsistent. Purpose of this study is to evaluate the prevalence and the effect on outcome of mutKIT in inv(16)(p13q22). Patients and Methods. 44 adults with inv(16) AML at diagnosis (median age 56 years, range: 17-88; M/F: 26/18), were centrally analyzed for mutKIT in exon 2, 8, 10, 11 and 17. Mutations were detected using sequencing and other sensitive assays such as ARMS (amplification refractory mutation system) PCR for D816V and with Tsp509I for N822K. Results. Data showed a prevalence of mutKIT mutation of 36.4% (16/44 patients). Among the mutKIT cases, we detected mutations in exon 17 (n=11), exon 2 (n=4) and exon 10 (n=1). Conclusion. Correlation between the KIT mutational status and median of WBC count at presentation: there was no significant difference between the mutKIT vs the unmutated (KIT-)

**CO-016**

**PREVALENCE AND PROGNOSTIC IMPACT OF KIT MUTATIONS IN ACUTE MYELOID LEUKAEMIA WITH INV(16)**


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**Introduction.** Several studies have recently pointed out the adverse impact of KIT mutations (mutKIT) on relapse incidence and overall survival (OS) in patients with t(8;21) AML. By contrast, the prognostic significance of mutKIT in patients with inv(16) is still inconsistent. Purpose of this study is to evaluate the prevalence and the effect on outcome of mutKIT in inv(16)(p13q22). Patients and Methods. 44 adults with inv(16) AML at diagnosis (median age 56 years, range: 17-88; M/F: 26/18), were centrally analyzed for mutKIT in exon 2, 8, 10, 11 and 17. Mutations were detected using sequencing and other sensitive assays such as ARMS (amplification refractory mutation system) PCR for D816V and D816H, enzymatic digestion with HINFI for D816V and with Tsp509I for N822K. Results. Data showed a prevalence of mutKIT mutation of 36.4% (16/44 patients). Among the mutKIT cases, we detected mutations in exon 17 (n=11), exon 2 (n=4) and exon 10 (n=1). Correlation between the KIT mutational status and median of WBC count at presentation: there was no significant difference between the mutKIT vs the unmutated (KIT-)

<table>
<thead>
<tr>
<th>NPM1+FLT3-ITD+</th>
<th>NPM1+FLT3-ITD-</th>
<th>NPM1-FLT3-ITD+</th>
<th>NPM1-FLT3-ITD-</th>
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<td>730</td>
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Figure 1.
Introduction. Cytokine induced killer (CIK) cells are CD3/CD56/CD8 positive cells which show considerable cytotoxic activity against leukemic cell lines and fresh samples and potent anti-tumoral activity in vivo in mice with little GVHD. We have investigated the cellular origin of CIK cells and tried to optimise their expansion in mice with little GVHD. We have investigated the cellular origin of CIK cells and tried to optimise their expansion in vitro for cell therapy purposes. Methods. CIK cells can be obtained in vitro by stimulation of mononuclear cells with IFN-gamma, followed by OKT3 monoclonal antibody and 21-28 days expansion in the presence of IL-2. Different cell populations were purified by FACS sorting and magnetic beads immunoselection and expanded in presence or absence of autologous irradiated mononuclear feeder cells. Results. In standard in vitro culture conditions starting from peripheral blood mononuclear cells (containing 0.5-6% CD3+CD34+ cells), we routinely observed a mean 665 (range 150-1477, n=8) theoretical fold expansion of CIK cells in 21-28 days and a mean 60% of CD3+/CD56+ (range 20-79, n=8). In an attempt to understand whether further expansion of CIK cells can be obtained with longer culture times, we continued to grow cells in IL-2 up to 42 days. These experiments showed that cell numbers reached a peak in 32-35 day and declined thereafter. In order to determine the origin of CIK cells, cell sorting and immunoselection experiments were performed demonstrating that they derive from the CD3+/CD56+CD8+ population. Indeed purification of CD3+/CD56+ cells at 15-21 days of culture and CFSE staining or cell counting showed that CIK cells are terminally differentiated and don't divide further. In contrast, the CD3+CD56+ cells are actively proliferating and can give rise to a new wave of CIK cells. We also investigated whether a restimulation at 20 days with interferon-gamma and autologous irradiated mononuclear feeder cells would allow greater and more sustained expansion of CIK cells. In these conditions we could indeed observe a second wave of CIK cells with a total fold increase of about 2000 at 42-45 days. These observations suggest the presence of CIK precursor cells even at the end of the standard 21 days culture which can still respond to appropriate stimulation. Discussion. These data demonstrate that cytotoxic CIK cells arise from CD3+CD56+/CD34- cells and also suggest the possibility to generate in vitro large number of CIK cells for clinical use by second stimulation in presence of feeder cells.

Isolation, Molecular and Functional Characterization of CD34− Leukemic Stem Cells in Chronic Myelogenous Leukemia (CML)

Fogli M,1 Manfredini R,1 Salvestrini V,1 Salati S,2 Bianchi E,2 Amabile M,1 Bertolini F,1 Tafuri A,1 Zini R,1 Testoni N,1 Rossi L,1 Martinelli G,1 Baccarani M,1 Ferrari S,1 Lemoli RM1

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We have previously characterized in normal human hematopoiesis a kinetically quiescent CD34− stem cell population, showing molecular and functional profiles different from that of CD34+ cells (Br J Haematol 2003; Stem Cells 2005). So far no, data are available on the presence of CD34− stem cells in leukemic hematopoiesis and their characteristics upon CD34+ acquisition. To this end, we sorted Lin-CD3-CD56−, Lin-CD3−CD34−, and Lin-CD3−CD34+ hematopoietic stem cells (HSCs) from 24 CML patients at diagnosis. The gene expression profile was studied using Affymetrix HG-U95Av2 GeneChip array. Molecular caryotyping and quantitative analysis of BCR/ABL transcript demonstrated that about one third of Lin-CD38−CD34− were leukemic. CML CD34− cells showed kinetic quiescence, limited clonogenic capacity and no long-term culture initiating cells (LT-CIC). However, stroma-dependent liquid cultures and cytokines induced CD34 expression on some HSCs, cell cycling, acquisition of clonogenic activity, generation of LT-CIC and increased expression of BCR/ABL positive CD34− cells showed an engraftment rate in NOD/SCID/beta2 null immunodeficient mice similar to that of CD34+ cells. At the molecular level, comparison analysis performed with Affymetrix GCOS software revealed 1907 genes differentially expressed in CML HSC subpopulations when compared to normal counterparts. Upregulated genes in CML samples were found in cell cycle, mitosis, DNA replication and DNA repair Gene Ontology (GO) categories, whereas immune response, defense response, antigen presentation and antigen processing GO categories were downregulated. Of note, HLA class I and II molecules were significantly downregulated, at the protein level in CML CD34− cells. Leukemic CD34− cells also demonstrated modulated expression of genes involved in apoptosis, like BCL2 family members, and malignant progression, like angiogenic cytokines. The analysis of transcription factor expression showed increased commitment to myeloid lineage and decreased self-renewal ability of CML cells. In conclusion, we identified in CML, for the first time, a quiescent leukemic stem cell subset devoided of CD34+ expression with peculiar molecular and functional characteristics which may be a potential target for CML therapeutics.
quences of 16±5%) only at day 3 after infusion of NK cells. In summar-
y, a two-step enrichment of CD34± NK cells allows the collection of a
suitable number of target cells to be used as adoptive immunotherapy
in AML patients. Infusion of cryopreserved NK cells is feasible and safe
and adoptively transferred NK cells can be detected in the peripheral
blood after infusion.

CO-020
THE ROLE OF CD34 ANTIGEN ON HUMAN HEMATOPOIETIC STEM CELLS
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CD34 is a highly glycosylated transmembrane protein, strongly
expressed on hematopoietic stem/progenitor cells (HSCs), but despite its
importance as a marker of HSCs, the function of this molecule remains
to be elucidated. Several studies suggest that CD34 may play a role in
cell adhesion and signal transduction on hematopoietic stem/progenitor
cells or mediate the interaction between HSCs and stromal lectins in the
bone marrow (BM). In order to better characterize the function of CD34
antigen on human hematopoiesic stem cells we examined both by small
interfering RNAs (siRNAs) and by retroviral-mediated over-expression,
the role of CD34 antigen in the regulation of hematopoietic stem/pro-
genitor cells lineage commitment. Here, we demonstrate that the expres-
sion of CD34 antigen is able to affect the differentiation capacity
of human HSCs, in particular clongenic assay and serum-free liquid cul-
ture show that the early down-regulation of CD34 enhances granulocy-
ic and megakaryocytic differentiation at the expense of the erythroid
one. In agreement with these results, microarrays analysis reveals that
siRNA-mediated CD34 gene silencing in CD34± HSCs induces the up-
regulation of genes involved in granulocytic and megakaryocytic com-
mitment and the down-regulation of the erythroid genes. In order to
confirm the results obtained by RNA interference, we overexpressed the
human CD34 cDNA in human CD34± cells by retroviral gene trans-
fer. CD34 transduced cells show a remarkable increase of erythroid pro-
genitors (BFU-E and CFU-E) and a dramatic decrease of granulocytic
(CFU-G) progenitors as evaluated by clonogenic assay. Furthermore,
immune phenotype analysis indicates that granulocytic differentiation
markers (CD66b and MPO) in CD34 transduced cells exhibit a decreased
expression; on the contrary, the expression of endothelial marker GPA is
up-regulated. Finally, the gene expression profile of CD34 transduced
cells show an increased expression of erythroid proteins versus controls.
Moreover, we find many markers of granulocytic differentiation such as
neutrophil and eosinophil granule proteins among down-regulated
genones. Altogether, these data indicate that CD34 antigen promotes the
differentiation of CD34± hematopoietic progenitors towards the ery-
throid differentiation pathway that is achieved, at least in part, at the
expense of other maturation lineages such as the granulocytic and
megakaryocytic ones.

CO-021
ENDOTHELIAL COLONY FORMING CELLS (ECFCs) FROM PATIENTS WITH CHRONIC
MYELOID LEUKEMIA, POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTEMIA, ORIGI-
NATE FROM A STEM CELLS LACKING THE DISEASE SPECIFIC MOLECULAR MARKER
Piaggio G,1 Corselli M,2 Bertolotti E,1 Parodi A,1 Chiavarina B,2 Sessarego M,1 Fugazza G,1 Garuti A,1 Bicaglupo A,1
PoZZi S,1 Frassoni P1
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oncologia, Ospedale San Martino, Genova, Italy

Background. The persistence of a common progenitor to haematopoietic
and endothelial lineage in the adult haematopoietic tissue appeared to
be validated when Bcr/Abl fusion gene was found in a variable propor-
tion of endothelial like cells generated in vitro from the bone marrow
and the peripheral blood of CML patients. However, the different cul-
ture conditions used to isolate and to expand endothelial cells (EC) in vitro
have generated some confusion. Only recently, the existence of two
EC populations has been clearly settled: colony forming unit-endothe-

tial cells (CFU-EC), are haematopoietic derived progeny committed to the
endothelial lineage while Endothelial Colony Forming Cells (ECFCs),
are vessel-forming progenitors. Aim. We have reconsidered the issue of
endothelial cell origin in CML (Chronic myeloid leukemia), PV
(Polyctemia Vera) and ET (Essential Thrombocytemia) to verify whether
circulating ECFCs isolated and expanded from the peripheral blood
of patients, bear the disease specific genetic marker of the leukemia clone
or whether it is restricted to the haematopoietic lineage. Methods. Forty-
three patients and 12 normal controls were included in this study. Twen-
ty-four samples were Philadelphia and Bcr/Abl positive CML, 19 were
MPDs, PV and ET with JAK2-V617F mutation. Peripheral blood
mononuclear cells were cultured in vitro and expanded cells were
searched for Bcr/Abl fusion gene by RQ-PCR and FISH, and for JAK2-
V617F mutation by nested PCR. Flow cytometry and capillary formation
assay in Matrigel were also performed. Results. due to their low frequen-
cy in the peripheral blood of patients, only 7/24 CML and 8/19 MPD
samples gave rise in vitro to a progeny of ECFCs. None of them carried
the disease specific genetic marker, they showed a complete outfit of
endothelial associated antigens and were able to form capillary like struc-
tures in vitro. Conclusions. differently from previous reports and based on
a clear distinction between ECFC and CFU-EC, this study shows, for the
first time, that ECFCs, derived from Ph-positive, Bcr/Abl positive CML
patients, lack the disease marker. In addition ECFCs from patients with
PV or ET lack the JAK2-V617F mutation. Thus it appears that in CML,
PV and ET the cell able to give rise to endothelial progenitors do not
derive from the malignant clone. This finding, per se, does not exclude
the possibility of a common ancestor cell for haematopoietic and
endothelial lineage but further suggests that hemangioblast in adult life
is likely to persist at a very low frequency, if any.

CO-022
PILOT STUDY OF SELECTION AND TRANSPLANTATION OF AUTOLOGOUS HIGHLY PURIFIED
CD133±STEM CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH ADVANCED
DISEASE
Isidori A,1,2 Motta MR,1 Tani M,1 Terragna C,2 Zinzani P,1 Curzi A,1 Rizzi S,1 Taioi S,1 Giudice V,1 D’Addio A,1 Gugliotta G,1 Conte R,1
Baccarani M,1 Lemoli RM1
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Objective. To assess the capacity of positively selected autologous
CD133± hematopoietic stem cells (HSCs) to reconstitute lympho-
myeloipoiesis in Chronic Lymphocytic Leukemia (CLL) patients under-
going myeloablative chemotherapy. Patients and Methods. Ten CLL
patients with advanced disease underwent uniform HSC mobilization
with chemotherapy and granulocyte colony stimulating-factor (G-CSF).
Positive selection of circulating CD133± HSCs was performed by
immunomagnetic technique. Highly purified HSCs were reinfused after
busulphan/melphalan myeloablative treatment. Results. A median num-
ber of 4.2±106 CD34± cells/kg and of 3.14±106 CD133± cells/kg were col-
lected. Immunomagnetic selection resulted in the reinfusion of a med-
ian number of 2.45±106 CD133± cells/kg (median purity: 94.8%; median
recovery 84%) and 2.4±106 CD34± cells/kg (median purity: 96%; med-
ian recovery 71%). HSC selection resulted in a median T-cell and
CD19±CD5± cell depletion of 3.85 log and 2.8 log, respectively. At the
molecular level, however, 8/9 evaluable purified HSC fractions were
contaminated by leukemic cells. All CLL patients showed rapid and sus-
tained myeloid engraftment after reinfusion of purified CD133± cells.
Of note, the immunological reconstitution did not significantly differ
from that routinely observed in patients reinfused with unmanipulated
leukapheresis products and no late infectious complications were
observed. Conclusions. Reinforcement of highly purified CD 133±
HSCs allows the rapid and sustained recovery of hematopoiesis after mye-
loablative treatment in advanced disease CLL patients although the purg-
ing potential of positive selection of CD133± cells is not optimal.
stromal cells might act not only supporting cell renewal exists in adult spleen and thymus. In cancer development, interacting with the immune system.

immunological sanctuary neoangiogenesis, but also creating an efficient, tissue-independent, MSCs from BM, spleen or thymus, did not lead to immediate loss of specific immune protection against tumor cells, but prevented memory anti-

Results. We found in both lymphoid tissues a reservoir of MSCs with the same immunophenotype (except CD106 expression) and differentiation potential of those derived from BM. Spleen- and thymus-derived MSCs displayed a great similarity with BM-derived MSCs in terms of modulation of T cell proliferation and cytotoxicity, although the suppressive activity of human spleen-derived MSCs at lower MSC/T cell ratios was less efficient as compared with BM- and thymus-derived MSCs. However, spleen- and thymus-derived human MSCs share the same mechanisms of inhibition (IFN-gamma and IDO-dependent) already shown in those derived from BM. In vivo immunization with s.c. injection of tumor cells together with MSCs from BM, spleen or thymus, did not lead to immediate loss of specific immune protection against tumor cells, but prevented memory anti-tumor immune response, thus determining tumor growth in normally resistant mice. 

Discussion. Our data suggest that a MSC pool for stromal cell renewal exists in adult spleen and thymus. In cancer development, stromal cells might act not only supporting tumor cell proliferation and neoangiogenesis, but also creating an efficient, tissue-independent, immunological sanctuary, which can prevent tumor rejection by interfering with the immune system.
ANEMIA - APLASIA - THALASSEMIAS

CO-025
PULMONARY HYPERTENSION (PH) IN PATIENTS WITH BETA THALASSAEMIA INTERMEDIA
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Pulmonary hypertension (PH), is a severe new emerging complication in thalassaemia intermedia patients (TI), during the third and fourth decades of life. In order to estimate the prevalence of this complication we evaluate by echocardiography the tricuspid gradient in a large cohort of patients referred to a single italian thalassaemia care centre. 115 subjects affected by TI, 51 males and 64 females, aged 39±9 years, were enrolled. The mean haemoglobin value was 9.0±1.1 g/dL. Only 30.4% (35/115) of patients entered late in life a regular transfusional regimen, 33.1% (38/115) were occasionally transfused while 34.8% (40/115) have never been transfused. 71% of the patients had undergone splenectomy and 24.3% (28/115) experienced at least one thrombotic event in their life, mainly portal vein thrombosis and deep vein thrombosis. All the patients underwent ECG record, ecochardiography, chest X-ray, abdomen ultrasound, heart MRI T2* and pulmonary function tests. PH was defined according tricuspid gradient as: mild-moderate (2.6-3.4 m/s) and severe (> 3.4 m/s). We expressed PH as tricuspid gradient, instead of pulmonary arterial pressure (PAP) because the technical variability is lower. PH mild to moderate was detected in 40.8% of TI patients (47/115) and severe in 3.5% (4/115). Patients with PH had lower haemoglobin levels and higher number of peripheral erythroblasts and platelets. Serum ferritin levels were higher in PH patients although they did not correlate with the presence and severity of PH (p=0.1590). Among patients with PH, 89% were splenectomized and 32% had previous thrombotic events, only one had ejection fraction <55%. Cardiomegaly was a common finding in TI patients with echocardiographic signs of PH. HcRT MRI T2* was within the normal range. A significant statistical difference in forced expired volume (FEV1) (p=0.0027) and forced vital capacity (FVC) (p=0.0361) between TI patients with PH and patients without PH was detected. TI patients with PH are older than patients without PH (p=0.0008). 3 out of 4 patients with severe PH were symptomatic and 2 of them started treatment with sildenafil. The major determinants for PH in thalassaemia intermedia seem to be age and splenectomy. Since thrombotic events are frequent in splenectomized TI patients due to a hypercoagulable status of thalassaemic red cells, we hypothesized that micro-thrombi of pulmonary small vessels could be at least in part responsible for PH in TI patients.

CO-026
NEED OF IMPLEMENTING THALASSAEMIA PREVENTION STRATEGIES AMONG IMMIGRANTS: 13 YEARS EXPERIENCE IN LATIUM AT CENTRO STUDI MICROCITEMIE ROMA (CSMR)
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In Latium CSMR has a long tradition in the management of thalassaemia. The successful prevention plan performed for more than 30 years is totally prospective and this drastically reduced the incidence of Thalassaemia Major and other haemoglobin disease among native people. But now we are in presence of a changing picture: the increasing number of foreign children affected by thalassaemia or other haemoglobin disorders among the multiethnic immigrants from endemics countries. These data are tending upwards and this condition is shared by a lot of industrialized areas of the world as a result of global population movements. Between 1994 and 2006 the amount of foreign patients visiting our structure moved from 2.7% to 7.24% of the total. Africa (29.8%) is the most represented ethnic group of the carriers or affected people, followed by East Europe (23.8%), Asia (22.0%), America (9.8%) and Australia (0.2%). 14.4% of the patients has uncertain origins. In the last 13 years we have examined 8074 foreign patients; 1907 of them were carriers or affected: 26.4% beta thal carriers, 42.5% suspect alpha thal carriers, 14.3% HbS carriers, 4.5% FBE carriers, 1.8% HBC carriers, 4% were carriers of rare Hb variants, 6.5% were affected. Since 1980 we have identified 50 foreign at risk couples: 23 were from East Europe, 20 from Africa, 5 from Asia, 3 unknown origins. 31 were retrospectives, while19 were prospective. The demographic transition that Latium has undergone over recent years is now responsible of a new reality: the incidence of Thalassaemia Major among natives diminishes; the number of non indigenous affected patients increases so much that since 2000 the value is nearly coincident with the number of the total new cases diagnosed for the first time. Until now the retrospective has been the most easily realizable method of prevention among multiethnic minorities now present in our region, which is the Italian area with the highest incidence of immigration. www.bld.info is a multi-lingual web site made by CSMR-ANMI ONLUS in order to reach and help all the foreign people still ignorant of their condition of carriers. Our challenge will consist in implementing health education and prevention strategies for thalassaemia and sickle cells disorders among immigrants. There is in fact a growing need of facilities to support patients care, carrier diagnosis, genetic counselling, throughout the region, to face this changing epidemiological picture.

CO-027
CIRCULATING T REGULATORY CELLS (TREG) ARE DECREASED IN PATIENTS WITH APLASTIC ANEMIA (AA)
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AA is a hematological disease characterized by an immune-mediated destruction of haematopoietic stem cells, leading to peripheral pancytopenia. Such immune pathophysiology is supported by several experimental data, as well as clinical observations; however initial pathogenic mechanisms are still debated. In recent years regulatory T cells (Treg) have been recognized as an integral part of the cellular immune system, engaged in the maintenance of immunological self-tolerance.
and immune homeostasis, including direct control of self-reactive immune responses; Treg have been found reduced in several autoimune diseases. Expression at very high level of the high affinity IL-2 receptor alpha chain (CD25) as well as the expression of the forkhead family transcription factor F3 (FoxP3), which is required for the development and function of these cells, has served as a phenotypic surface marker for this T cell subset. We investigated Treg level in a cohort of AA patients (n=21) and healthy volunteers (n=15); flow cytometry identification of Treg was obtained by surface and intracellular staining of PBMCs for Treg markers (CD3, CD4, CD25, FoxP3), using a combination of monoclonal antibodies. PBMCs were initially gated according to physical features, CD3 and CD4 expression; then, based on CD25 expression, the CD4+ T cells were subdivided into CD25-, CD25int and CD25hi, and the co-expression of CD25hi and Foxp3 was determined. We initially show that untreated AA patients (n=12) have less CD4+CD25hi T-cells in comparison to controls, as expressed by both frequency on PBMCs and absolute number (median 0.07 vs 0.21 and 1.06 vs 4.76 as percentage and absolute number; p=0.03 and p=0.003). Treg cells seem to be even lower in patients with active disease if the partner is a carrier for beta thalassemia and particularly in areas with high prevalence and heterogeneity of beta thalassemia.

**CO-028**

**GENOTYPE-PHENOTYPE CORRELATION IN HBA2 BORDERLINE SUBJECTS**

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The heterozygous states for beta thalassemia show wide phenotypic diversity. Usually the presence of a single beta thalassemia allele is associated with hypochromic microcytic red cells and an elevated level of HbA2 (>3.1%). The last index is most typical marker to define the state of beta thalassemia carrier but, in some cases, the effect of a single beta thalassemia allele can be completely silent with normal haematological indexes and normal or borderline HbA2 and HbF levels so that some individuals may be missed during screening program for beta thalassemia.

A retrospective study was conducted on 23485 HB analysis done during program for beta thalassemia carrier screening in the Sicilian population. 3934 (16.75%) subjects showed HbA2 levels between 3.1% and 5.9% with absence of common HB variants and iron-anemia; molecular analysis was conducted on 410, partners of carrier subjects or couple with both partners showed borderline value of HbA2. The aim of this study was evaluate the correlation between HbA2, borderline levels and molecular defects of globin genes and to obtain a predictivity data for the identification of subjects carriers of thalassemia with these phenotypes. 94 subjects (22.9%) resulted positive for a molecular defect in the beta, delta or in the alpha globin genes while none molecular defects were found in the remaining 316 individuals. Considering MCV value, 84 samples (29.5%) showed an MCV<79.9 fl and 55 (65%) resulted positive for mutations versus 29 negatives while among the 326 (79.5%) samples with MCV>80 fl, just 39 (11.9%) were positive versus 287 negative. The most present molecular defect was the beta IVS 1 nt 6 (25.5%), followed by beta globin gene promoter’s mutations -101 and -92 (21.5%), triple alpha anti 3.7 (15.9%), alpha mutations (12.8%), co-eredit of beta and delta mutations (11.7%), variants and nucleotides ambiguity in the beta globin gene (6.4%). In conclusion, in a screening for beta thalassemia, subjects with HbA2 derangement must be always investigated using particular attention especially if the partner is a carrier for beta thalassemia and particularly in areas with high prevalence and heterogeneity of beta thalassemia.

**CO-029**

**MULTICENTRIC RETROSPECTIVE STUDY ON ERYTHROXCHANGE IN SICKLE CELL DISEASE. PRELIMINARY RESULTS**

Vassanelli A,1 Lodi G,2 Forni G,3 Cabibbo S,4 Pennisi A5 Perseghin P6 De Meis I,7 Messina R8 Pani M,9 Guastella G10 Lunghi M11 Mazzoni A12 Capuzzi E13 Dentì A14 Ferrini C15 Sassi M16 Mariano MT17 Dal Canton18

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Introduction. Sickle cell disease (SCD) is a relatively common haemoglobinopathy, characterized by chronic haemolytic anemia and episodic occurrence of vaso-occlusive events that precipitate acute painful episodes. The therapy consists of symptomatic measures like giving analgetics, decreasing blood viscosity, chronic transfusion therapy or erythroxchange (EEX), frequently used either therapeutically or electively, in order to prevent major complications (pregnancy, perioperative management, patients with >3 acute crisis/year). In a EEX, the patient’s red cells are removed and replaced by exogenous normal red cells, decreasing the percentage of sickle hemoglobin (HbS) and maintaining a net balance in iron accumulation, that is an important benefit of EEX versus simple transfusion. Methods and Results. We present the preliminary results of a retrospective multicentric study about use of EEX in treatment of patients with sickle cell syndrome. See Table 1.

**Table 1.**

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<th>Paediat.</th>
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**Discussion.** This preliminary results confirms that EEX is an effective...
therapy for acute and chronic complications of SCD, both in adult and pediatric patients, even if only in few hospitals pediatric patients are treated with EEX. A great number of patients are non-Italian (from Africa or East-Europe), suggesting an increasing medical and social problem. 

Conclusions. In treatment of SCD, EEX, performed using modern cell separators, is an effective and safe therapy (more than 2900 procedures are performed in Italy until 2006, without major side effects), but is probably underutilized. A Multidisciplinary Collaborative Group is recently created by Scientific Societies (SIMTI, SIDEM, SOSTE) in order to collect data, to share experiences, to define protocols and long term follow-up patients observing results, treatment efficacy, occurrence of side effects, and to optimize treatment and improve patient’s quality of life.

CO-030

IMPACT OF CYTOKINE GENE POLYMORPHISMS IN APLASTIC ANEMIA (AA)

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A is an uncommon hematological disease characterized by a severely depressed marrow cellularity leading to pancytopenia. In most cases, AA results from immune-mediated inhibition of hematopoietic stem cells, as supported by several experimental data as well as by clinical results following immunosuppression (IS). The pivotal player of the immune system are cytokines, which are potent immunomodulatory molecules acting as mediator of inflammation and immune response. Cytokine production is under genetic control; indeed, their genes present polymorphisms at specific sites, potentially affecting the overall expression (i.e., those located within coding/regulator regions). Thus, such polymorphisms may be associated with autoimmune conditions. We studied immunogenetic background in a cohort of 40 AA patients, by single nucleotide polymorphisms (SNPs); 60 healthy donors matched for ethnicity were analyzed as a control. The following SNPs were analyzed: IL-1a (-889 T/C), IL-2 (-330 T/G +146 G/T), IL-4 (-1998 T/G -890 T/C +33 T/C), IL-1B (-1700 C/T), IL-1RA (masp111110 T/C), IL-4RA (+190 G/A), IL-1B (+511 C/T, +3962 T/C), IL-6 (-174 G/C, nt565 G/A), IL-10 (-1082 G/A, –819 C/T), IL-12 (-1158 C/A), TGF-β (+10 C/T, +45 G/C), INF-y (+874 A/T), TNF-α (+308 G/A, –238 G/A). No significant difference in AA cohort was found for the SNPs in IL-4Ra, IL-12, IL-1B, INF-γ, IL-2, IL-1α and TNF-α. However, when we examined the frequency of TGF-β genotypes, increased frequency of GG and CT genotypes was found in the AA population. In addition, we found a lower incidence of TT genotypes for the SNPs in IL-4Rα (p=0.003), consistent with high secretory phenotype, was found in the AA population. In addition, we found a lower incidence of TT genotypes for the SNPs in IL-4Rα (p=0.003), consistent with high secretory phenotype. In sum, our findings demonstrate that a specific combination of cytokine genotypes might act together to predispose to AA, leading to specific disease presentation. More extensive profiling based on these and other cytokine gene polymorphisms could aid in determining best candidates for immunosuppressive therapies.

CO-031

CARDIAC IRON EVALUATION BY T2* MRI IN TRANSFUSION DEPENDENT PATIENTS WITH ACQUIRED ANEMIAS

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The aim of our study was to evaluate cardiac iron in adult patients with transfusional iron overload caused by acquired anemias, in particular in patients affected by myelodysplastic syndromes. Therefore we measured cardiac MRI (Magnetic Resonance Imaging) T2* in 25 consecutively studied patients, by using standard protocols for cardiac MRI, and assessed the correlation between T2* values and cardiac iron parameters. The following parameters were assessed: patient’s age, sex, body surface area, hemoglobin and hematocrit levels, transfusion rate, serum ferritin levels, and T2* values. The results of our study showed a significant correlation between serum ferritin levels and T2* values (R=0.70; p=0.001). The patients with cardiac T2* value > 20 milliseconds revealed signs of cardiac failure in one year of follow up. No patient who had received less than 100 transfusions (excluding patient with genetic alterations) presented myocardial iron deposition as documented by a T2* superior to 20 milliseconds. Cardiac T2* was weakly correlated with hepatic T2* (R=0.23; p=0.04). None of the patients with hepatic T2* superior to 2.5 milliseconds, expression of moderate hepatic iron overload, showed myocardial iron deposition. No correlation was found between serum ferritin level and myocardial iron. (R=0.396; p=0.093). Myocardial iron deposition develops after severe hepatic iron deposition has occurred in unchelated adult patients with acquired anemias.

CO-032

SUBCUTANEOUS ALEMTUZUMAB IS SAFE AND EFFECTIVE FOR TREATMENT OF SEVERE APLASTIC ANEMIA (SAA) OR LINEAGE-RESTRICTED APLASIA: A PILOT STUDY

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Acquired marrow failure syndromes may affect all hematopoietic lineages, as in SAA, or may selectively involve single lineages, as in pure red cell aplasia (PRCA) or in agranulocytosis (AGR). All these conditions are associated with autoimmune conditions. We studied immunogenetic background in a cohort of 40 AA patients, by single nucleotide polymorphisms (SNPs); 60 healthy donors matched for ethnicity were analyzed as a control. The following SNPs were analyzed: IL-1a (-889 T/C), IL-2 (-330 T/G +146 G/T), IL-4 (-1998 T/G -890 T/C +33 T/C), IL-1B (-1700 C/T), IL-1RA (masp111110 T/C), IL-4RA (+190 G/A), IL-1B (+511 C/T, +3962 T/C), IL-6 (-174 G/C, nt565 G/A), IL-10 (-1082 G/A, –819 C/T), IL-12 (-1158 C/A), TGF-β (+10 C/T, +45 G/C), INF-y (+874 A/T), TNF-α (+308 G/A, –238 G/A). No significant difference in AA cohort was found for the SNPs in IL-4Ra, IL-12, IL-1B, INF-γ, IL-2, IL-1α and TNF-α. However, when we examined the frequency of TGF-β genotypes, increased frequency of GG and CT genotypes was found in the AA population. In addition, we found a lower incidence of TT genotypes for the SNPs in IL-1RA (p=0.003), consistent with high secretory phenotype. In sum, our findings demonstrate that a specific combination of cytokine genotypes might act together to predispose to AA, leading to specific disease presentation. More extensive profiling based on these and other cytokine gene polymorphisms could aid in determining best candidates for immunosuppressive therapies.
CONFERENCE ABSTRACTS

ALLOGENEIC TRANSPLANTATION

**CO-033**
A PROSPECTIVE PHASE II STUDY ON TANDEM AUTOGRFTING-NONMYELOGBLATIVE ALLOGRAFTING FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: FINAL RESULTS OF A MULTICENTER GITMO (GRUPPO ITALIANO TRAPIANTO MIDOLLO OSSEO) PROTOCOL

Sorasio R,1 Patriarca E,2 Giaccone G,1 Mattei D,1 Allione B,1 Carnevale-Schianca E,1 Rambaldi A,2 Casini M,2 Montefusco V,2 Parma M,1 Bavaro P,1,2 Onida E,1 Bosca A,2 Castagna L,3 Iori AP,4 Rotta M,1 Fiore F,2 Benedetti E,1 Mordini N,1 Massaia M,1 Palumbo A,1 Aglietta M,1 Lewis A,1 Foà R,1 Di Bartolomeo P,10 Pogliani E,1 Lambertenghi-Deliliers G,11 Falda M,12 Petriti M,12 Corradini B,1 Fanin R,2 Ricardi U,16 Ciccone G,11 Baldi L,1 Bruno B,1 Boccadoro M1

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Introduction. Despite remarkable recent advances in the treatment for multiple myeloma, allografting remains the only potential cure in a subset of patients. The development of nonmyeloablative conditioning has reduced the transplant-related mortality and extended the eligible age for transplantation up to 65-70 years. However, prior cytoreductive high-dose chemotherapy appears to play a key role in the achievement of high response rates. Methods. From January 2000 to June 2005, 106 newly diagnosed patients younger than 65 years were enrolled in a prospective multi-center study. Briefly, first-line treatment plan included vincristine, adriamycin and dexamethasone (VAD)-based induction chemotherapy, a cytoreductive autograft with melphalan at 200 mg/m² followed by non-myeloablative total body irradiation and allografting (Tandem auto-allo) allowing allografting all patients readily promptly achieved graft-vs-host disease (GVHD) prophylaxis included cyclosporin and mycophenolate mofetil. One-hundred-two patients, median age 54 years, (range 30-65,) Four patients did not completed the program whereas 4 because of consent withdrawal and were excluded from the analysis. Primary objective was to evaluate the overall survival (OS) and event-free survival (EFS) survivals from diagnosis, and transplant related toxicities. Results. A follow-up of 34 ± 14 months, we obtained a disease response, including one CR. Discussion. Tandem auto-allo allows long-term disease control especially in patients with reduced tumor burden at the time of allografting. Intensified pre-transplant cytoreduction with new drugs combined with post-transplant graft-vs.-myeloma effects may further improve the clinical outcome.

**Figure.**

**CO-034**
NKGD2-MEDIATED INDUCTION OF NK ACTIVITY BY ALLOGENIC CD34+ BLOOD CELLS.

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Background. We have previously demonstrated that human CD34+ cells and allogeneic T lymphocytes cross-interact in vitro, resulting in T cell proliferation and CD34+ cell differentiation into dendritic cells. In this study, we addressed the hypothesis of a cross talk between CD34+ cells and allogeneic NK cells. Methods. Alloreactive NK cell donors were selected based on NK-permissive HLA C mismatch. Immunomagnetically purified CD34+CD56+ NK cells were cultured with purified allogeneic CD34+ or CD14+ blood cells or with 1000 U/mL IL-2. Following culture, NK cells were used to lyse NK-sensitive K562 (NK activity) or NK-resistant Daudi cells (LAK activity) in a standard 1Cr release assay. Results. CD34+ cells induced greater NK activity than monocytes (50±14% vs 15±17% lysis of K562 cells, respectively) (p=0.02). While LAK activity was negligible before culture, it increased upon culture with CD34+ cells, as opposed to monocytes (53±11% and 22±16%) (p=0.01). Stimulation with CD34+ cells was comparable to high dose IL-2. NK cell activation was also documented by the upregulation of CD69 and ULBPs in CD34+CD16+ NK cells. Conclusions. Our study suggest a cross talk between CD34+ cells and NK cells, which may provide a novel candidate for NK cell therapy.
CO-035
THIOTEPA BASED CONDITIONING REGIMENS FOR ALLOGENEIC STEM CELL TRANSPLANTS: OUTCOME IN 374 PATIENTS - WITH A MEDIUM AGE OF 48 - GRAFTED FROM RELATED OR UNRELATED DONORS

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Background. Following the experience of the Perugia group, who first introduced thiopeta (THIO) in allogeneic stem cell transplanted (HSCT), we have developed several conditioning regimens, including THIO , cyclophosphamide (CY), Fludarabine (FLU), melfalan (MEL) and TBI mainly for patients above the age 45. Aim of the study. Assess the outcome of patients undergoing an allogeneic HSCT with a THIO based conditioning regimen. Patients. 374 patients were allografted with a THIO based regimen, between 1994 and 2005, from HLA identical siblings (n=221) or family partially mismatched (n=67) or unrelated (n=86) donors. Median patient age was 48 years (range16-67). The stem cell source was unmanipulated in all cases , either bone marrow (n=276) or peripheral blood (n=96). The conditioning regimens were classified as reduced intensity (n=177) (THIO+CY or THIO+FLU) or intensified (n=197) (THIO +CY with MEL or TBI 200r). The disease was in 1stCR (n=221) or more advanced phase (n=153). Diagnosis were as follows chronic myeloproliferative disease (n=123), acute leukemia (n=120), myelodysplasia (n=46), other (n=65, including lymphoma and myeloma). All patients received cyclosporin methotrexate GvHD prophylaxis. Alternative donor transplants received additional anti-thymocyte globulin in the conditioning. The median follow up for surviving patients is 5 years (range 1-12 years). Results. The overall actuarial 10 year survival is 40% (60% vs 80% in CR1 or >CR1 disease). The cumulative incidence (CI) of transplant related mortality (TRM) at 10 years is 29% (18% vs 36% for CR1 or >CR1 disease). TRM for CR1 patients grafted from identical siblings (n=94) is 12%. Acute GvHD grade III-IV was seen in 6% of sibling HSCT and 12% of alternative donor grafts. The CI of relapse related death (RRD) at 10 years was 27% (18% vs 32% in CR1 or >CR1). There was no effect of patient age, nor of stem cell source on survival. In multivariate analysis on survival, significant predictors were disease phase (RR 2.4 of death for patients beyond CR1) and intensity of the conditioning (RR 1.66 for intensified regimens). These two variables (disease phase and intensity of the conditioning regimen) produce encouraging long term survival, with a low incidence of GvHD and low toxicity alone, especially in patients with early disease. The addition of MEL or TBI reduces RRD, but increases significantly TRM and does not improve survival. Disease phase remains a major predictor of outcome.

CO-036
LONGITUDINAL FOLLOW-UP OF WT1 GENE EXPRESSION AS MONITORING OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION

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Introduction. WT1 is identified as a tumor suppressor gene encoding a transcriptional regulator and playing a role in the development of Wilms Tumor. WT1 overexpression is described in several oncological diseases, including leukemias. The majority of acute myeloid leukemia (AML) patients don't have a suitable specific molecular marker for monitoring minimal residual disease (MRD). Quantification of WT1 in bone marrow samples can be useful as a marker of MRD and can predict the relapse of AML. Methods and Results. Here we report the preliminary results of our study aimed to evaluate the dynamic expression of WT1 in AML patients after allogeneic bone marrow transplantation (BMT). The expression of WT1 was measured at selected intervals of treatment, using Real Time Quantitative RT-PCR with the specific TaqMan probe; the WT1 expression was related to the control gene ABL. The cDNA level of WT-1 was detected in bone marrow samples from 30 AML pts (18 males and 17 females) at diagnosis, at the time of transplant and after the allogeneic BMT. All cases showed high WT1 expression levels at diagnosis with a mean of 4648 (SD 3847) and a median of 3679 (range 658-13923) copies WT1/10000 Abl. At transplant 21 pts (70%) were in complete cytologic remission (CcR) and 9 (30%) had refractory or relapsed AML. Bone marrow samples from pts in CcR at BMT showed significantly lower WT1 expression levels (mean 114±150), compared to the samples from pts with relapsed or refractory disease (mean 4544±8347) (p=0.004). After BMT a rapid decline of WT1 expression levels was observed in all pts that attained or maintained a condition of CcR (Figure 1A). After a median follow up of 7 months from transplant, 4 out 30 pts relapsed (13%) and all of them had an increase in WT1 expression (Figure 1B). Two of these pts died with leukemia and two were successfully reinduced with DLI chemotherapy with a rapid reduction of WT1 levels. Besides we found a concordance between WT1 expression levels and other disease markers (when available).

Figure 1A. WT1 after BMT in pts in CcR.

Figure 1B. WT1 in 4 relapsed pts post BMT.

Conclusions. In our preliminary experience there was a complete concordance between WT1 expression levels (measured by quantitative RT-PCR) and status of disease before and after BMT. WT1 (from bone marrow samples) may be used as a non-specific leukemia marker (NSLM) for monitoring MRD and as a predictor of AML clinical relapse. Based on this results cases with a rapid increase of WT1 levels after BMT and without GvHD should be candidate to discontinuation of immunosuppressive therapy and/or DLI.
CO-037
DIRECT INTRA BONE INJECTION OF UNRELATED CORD BLOOD CELLS OVERCOMES THE PROBLEM OF DELAYED OR FAILURE TO ENGRAFT AND IMPROVES THE FEASIBILITY OF HEMATOPOIETIC TRANSPLANTATION IN ADULT PATIENTS

Ibaciti A,1 Raiola AM,2 Guandalini E,2 Sassareno N,1 Parodi A,1 Pozzi S,1 Pinto V,1 Corcelli M1,3 Podestà M1,4 Piaggio GF1,4 Gobbi MF1,4 Baccigalupo A1,4 Frassoni F1
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Background. Cord blood transplants (CBT) are associated with delayed or failed engraftment in a significant proportion of patients (pts). Two previous our observations suggested (i) that, in the animal model, direct intra-bone (i.b.) injection improves seeding efficiency and (ii) that the intra-bone route might be responsible for robust hematological recovery also when low numbers of HLA matched CB cells were transplanted.

Methods. Unrelated CB (4/6 or 5/6 HLA antigen matched) were selected for 20 consecutive pts. Median transplant cell dose was 2.7×10^7/kg (range 1.6-4.2). CB cells were concentrated in 4 syringes of 5-6 ml each and infused in the supero-posterior iliac crest (SPIC) under rapid general anesthesia (10 min. with propofol). Pts. median age was 58 years (18-85), 16 had acute leukemia, 2 chronic myeloid leukemia , 2 Hodgkin disease. Sixteen pts. had refractory or advanced disease, whereas 4 had high risk first remission leukemia. Most pts. (n=16) were prepared with conventional CY-TBI. Results. The infusion of cells i.b. in SPIC (some pts bilaterally; some monlaterally) was uneventful. Three pts. are not evaluable because died within day 10 from transplant. All pts. surviving more than 10 days engrafted (100%). Median for PMN engraftment (≥0.5×10^9/L) was day 25 (14-40), whereas for platelets (≥20×10^9/L) it was day 36 (22-60). Two pts. relapsed and three died of infection. Twelve pts. are alive and well in hematologic remission at a median follow up of 8 months (range 4-13). 100% donor chimerism was documented since 30 days onward after transplant bilaterally. From day +30, CFC progenitors the early and committed hematopoietic compartment had already seemed already to be replenished since CFC and LTC-IC reached the lower values of the range of normal individuals range of normal individuals (median CD34+ cells 4.1 (2,5-7,5)×10^6/kg). Only 3 pts experienced Acute GVHD (2 grade I and 1 grade II). Interestingly, injected T cells after i.b. injection come immediately in contact with mesenchymal stem cells (MSC) and osteoblasts, known to be potent immunosuppressants. Conclusions. This study has shown that intra-bone route of administration is responsible for robust hematological recovery also when low numbers of HLA mismatched CB cells are transplanted. Nearly all patients for whom a CB unit was searched were able to undergo CBT. This may change our policy of hematopoietic cell transplants.

CO-038
DONOR-RECIPIENT STATUS FOR HLA CLASS I LIGANDS AND NOT THE KIR GENOTYPE, IS PREDICTIVE OF UNRELATED HSCT OUTCOME IN BETA-TALASSEMIA PATIENTS

La Nasa G,1 Littera R,2 Ledda A,1 Piras E,1 Vaccia A,1 Caocci G,1 Pizzati A,1 Arras M,1 Floris R,1 Giardini C,1 Locatelli F1 Carcassi C2
1Cattedra di Ematologia, Centro Trapianti di Midollo Osseo, Ospedale Binaghi, Cagliari; 2Cattedra di Genetica Medica, Università di Cagliari, Cagliari; 3Divisione di Ematologia, CTMO, Ospedale di Pesaro, Pesaro 4Oncoematologia PREDICTIVE OF UNRELATED HSCT OUTCOME IN BETA-THALASSEMIA PATIENTS

Pizzati A,1 Arras M,1 Floris R,1 Giardini C,3 Locatelli F,4 Carcassi C2
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Background. Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for the majority of patients with PNH. Allogeneic HSCT may cure PNH. In this retrospective study we report the results of allogeneic HSCT in 23 patients (14 males and 9 females) affected by PNH who were transplanted in 8 Italian institutions between July 1988 and October 2006. The median age at time of HSCT was 30 years (22-60). The median time from diagnosis to HSCT was 31 months (3-208). All patients had received various treatments before HSCT including steroids, immunosuppressive drugs and growth factors. Nineteen patients were transfusion-dependent. The median number of packed red blood cells and platelet concentrates received before HSCT was 30 (4-500) and 22 (6-86) respectively. At time of HSCT, the median peripheral hematological counts were: polymorphonucleates (PMN) 1750 (20-10240)×10^9/L, hemoglobin 8,7/g/dl (6,9-11), platelets (PLT) 78 (6-855)×10^9/L. Two patients were severely pancytopenic at time of HSCT. Twenty-one patients were transplanted from HLA identical siblings and 2 from matched unrelated donors. The donor’s median age was 32 years (20-50). The conditioning regimen was myeloablative for 15 patients (Busulfan and Cyclophosphamide). Eight patients received a reduced intensity conditioning including Fludarabine, Cyclophosphamide, Melphalan and Total Body Irradiation. As of May 2007, 17 patients are alive and well in hematologic remission at a median follow up of 107 months (6-210). The 10-year Kaplan-Meier probability of disease-free survival is 70%. This study confirms that HSCT is a curative treatment for the majority of patients with PNH.
SUCCESSFUL GRAFT-VERSUS-HOST DISEASE (GVHD) PROPHYLAXIS IN UNMANIPULATED BONE MARROW TRANSPLANTATION (UBMT) FROM HAPLOIDENTICAL RELATED DONOR FOR HEMATOLOGICAL MALIGNANCIES


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In this pilot study we evaluated the feasibility and clinical value of an intensive regimen for GVHD prophylaxis in UBMT from HLA-haploidentical related donor. Twenty-two patients with a median age of 28 years (12-59) were transplanted for AML (n=12), ALL (n=5), MDS (n=1), CML (n=2), Hodgkin lymphoma (n=1) and plasmacell leukemia (n=1).

Ten patients were in first complete remission and 12 were in more advanced phase of their disease. All donors were HLA identical at 1 haplotype and mismatched for 2 or 3 loci on the unshared haplotype. The conditioning regimen consisted of Cytarabine 3 g/m²/d for 3 days and Cyclophosphamide 45 mg/Kg/d for 2 days combined with 10 Gy fractionated-total body irradiation (n=5), or Trensulfan 14 g/m²/d for 3 days (n=9) or i.v. Busulfan 3.2 mg/Kg/d for 3 days (n=6). Because of graft failure following a double haploidentical T-cell depleted or an autologous PBSC transplant, 2 patients were prepared with Fludarabine alone 40 mg/m²/d for 3 days. All patients received Fresenius anti-thymocyte globulin at 5 mg/Kg/d from day -4 to -1. As GVHD prophylaxis, the patients received Cyclosporine i.v. at 1.5 mg/Kg/d from -7 to -1 and 3 mg/Kg/d from 0 to +28 and orally at 5 mg/Kg/d from +29 until +365; Methotrexate 15 mg/sqm on day +1 and 10 mg/sqm on day +3, +6 and +11; Mycophenolate mofetil 1 g/d from day +7 to +100; Basiliximab 20 mg i.v. on day 0 and +4. Donors were primed with Filgrastim at 3-4 microg/Kg/d from day -7 to -1. Bone marrow cells were infused fresh and unmanipulated. The median dose of nucleated cells, CD34+ and CD3 cells was 6.95x10^6/kg (1.01-14.4), 2.3x10^7/Kg (1.77-8.2) and 30.9x10^6/Kg (16.9-74.6) respectively. All patients engrafted with a median time of 16-21 days to reach >0.5x10^9/L neutrophils and 20-30,000 x 10^9/L platelets. The median time to reach >0.5x10^9/L platelets. The median time to reach >0.5x10^9/L platelets.

Acute GVHD was grade 0 in 12 patients, grade I in 5 and grade II in 4. No patient had evidence of chronic GVHD.

The transplant related mortality at 6 months was 27%. Five patients died of infection and 1 of neurological complications between the median follow-up of 140 days (30-706). The GVHD prophylaxis described in our study seems to be very effective in the setting of the haploidentical UBMT. The results are particularly encouraging and deserve further study with more patients and longer follow-up.

CO-041

DIFFERENT PROGNOSTIC GROUPS OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS DEFINED BY CD38 AND ZAP-70 EXPRESSION, IG VH GENE STATUS


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Background. In chronic lymphocytic leukemia (CLL) biologic risk factors such as IgVH gene status, CD38 and ZAP-70 expression have been identified, but the relative prognostic impact of the individual parameters needs to be further investigated. Aims. The purpose of this study was to assess the individual predictive power of the VH mutational status, CD38 and ZAP-70 expression with respect to time to treatment (TTT) in a cohort of 390 B-CLL. Moreover, in order to better define at molecular level the different prognostic categories, we performed gene expression profile in a representative panel of samples. Patients. The median age of the patients was 65 years; 39% were females; 85% were classified as Binet stage A, 9% stage B and 6% stage C. Neoplastic CD19+ cells were highly purified by negative selection and evaluated for ZAP-70 and CD38 expression and IgVH status. In particular, ZAP expression was investigated by Western blot and cases were classified as ZAP-70high, ZAP-70weak and ZAP-70neg. Sixty patients included in the study and representative of the different prognostic variables, were profiled with gene expression U133A Array and the transcriptional patterns were investigated by supervised analyses. Results. First, we evaluated the prognostic significance of the CD38 expression. Since different cut-off points of the CD38 expression (7%, 10%, 20%, and 30%) have been proposed for discriminating different prognostic groups, we calculated the TTT curve at several cut-off values. Although all of them were capable of individuating CLL cases with statistically significant different TTT, the highest log rank was observed with a cut-off of 50% (3-years TTT 88% for CD38<30% versus 50% for CD38>30%, p<0.0001). When CLL cases were stratified according to the ZAP-70 expression levels, the 3-years TTT probability was significantly longer (p=0.0001) in ZAP-70neg cases (92%) compared with ZAP-70 weak (83%) and ZAP-70 strong (52%). With regard to the IgVH gene status (considering the classical VH homology cut-off value of 98%) a statistically significant predictive power on TTT was observed (3-years TTT probability: IgVH mutated versus IgVH unmutated: 91% versus 42%; p<0.0001). Among other clinical and haematological parameters, only the Binet staging showed a predictive power. Finally, all the above mentioned variables still maintained an independent prognostic impact at Cox multiple regression model (Binet stage: Exp(B) 2.7, p<0.0001; IgVH: Exp(B) 0.9, p=0.002; ZAP-70 Exp(B) 1.5, p=0.019; CD38 Exp(B) 1.1, p<0.0001). Specific transcriptional patterns were identified by supervised analyses in patients stratified according to the defined cut-off levels for each of the three prognostic variables. Notably, with regard to CD38 and ZAP, significant expression profiles were found only in patients with CD38 more >80% and ZAP strong, supporting the significance of these cut-offs for the risk-assessment. Conclusions: This study showed: 1) the best cut-off value of CD38 expression for individualizing CLL cases with statistically different TTT was 30%; 2) all the three biologic risk parameters were significantly related to TTT at univariate analysis; 3) the multiple regression model of Cox showed that all 3 parameters have an independent prognostic impact on TTT.
HAIRY CELL LEUKEMIAS (HCL) WITH UNMUTATED IGHV GENES IDENTIFY THE MINOR SUBSET UNRESPONSIVE TO SUBCUTANEOUS SINGLE AGENT CLADRIBINE


Hairy cell leukemia (HCL) is generally responsive to single-agent cladribine and only a minority of patients are refractory and with poor prognosis. HCL have mutated (M-HCL) and, in a minority of patients, unmutated immunoglobulin heavy chain variable region (IgHV)-genes (U-HCL). In an ongoing randomized multicenter clinical trial (ICGH-CL2004) evaluating efficacy and toxicity of subcutaneous cladribine (Ltkap, Lipomeds, CH) given 0.1 mg/kg/die for 5 or 7 consecutive days in newly diagnosed patients we prospectively investigated 1) toxicity and response to treatment and 2) clinical and molecular indicators of response. Among molecular parameters, the expressed tumor IgHV genes were investigated by RT-PCR/sequencing. Forty of 44 patients responded to subcutaneous cladribine (27 CR, 13 PR), while 4/44 patients demonstrated refractory or progressive disease, indicating activity of subcutaneous cladribine similar to that reported with standard intravenous administration. Responses and toxicity appeared independent of treatment schedule. Search of indicators of response revealed that leukocytosis and large splenomegaly indicated poor response to subcutaneous cladribine (2-tail Fisher’s Exact Test p-value=0.005 and p=0.009, respectively). Tumor IgHV gene was identified in 27 patients. Twenty-three HCL had mutated tumor IgHV (homology to closest germline IgHV 95%) while 4 had unmutated IgHV-gene (homology 99%). Most remarkably, the 4/4 refractory HCL only expressed unmutated IgHV-genes while 4 had unmutated IgHV-genes (homology?98%). Most patients with a longer median follow-up, the high percentage of patients with a longer median follow-up, the high percentage of patients

Overall survival was 60% after a median follow-up of 24 months (range 1–48). Survival was significantly associated to response achievement, being 77.3% in responders 38.8% in non responders (p=0.006). The cause of death was infection, mostly in relapse or progressive disease. Pneumocystis pneumonia was documented in two patients as late infection (nine months after the end of Cam). Low-grade intermittent fever was observed in 17.5% of the patients, Grade 3-4 neutropenia in 32.5%; Grade 4 thrombocytopenia in 2.5%; Grade 3 anemia in 5%. Overall 35% of the patients had complications of proven or suspected infective origin. Transient CMV reactivation without clinical disease was documented in 8 patients, and treated successfully with valganciclovir. Provided an accurate prophylaxis and/or pre-emptive therapy was carried out, Cam was safely administrated to HBV positive patients. We confirm on a larger number of high-risk relapsing/refractory CLL patients with a longer median follow-up, the high percentage of response, long remission duration, and the favourable toxicity profile of low-dose subcutaneous cladribine (Cam), already shown in our pilot study (Cortezielli A et al. Haematologica 2005).

Table 1. Response (%) in relation to patients characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>OR</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n=40)</td>
<td>55.0</td>
<td>27.5</td>
<td>23.1</td>
<td>32.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Failed fludarabine (n=25)</td>
<td>56.0</td>
<td>28.0</td>
<td>28.0</td>
<td>32.0</td>
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</tr>
<tr>
<td>Failed rituximab (n=13)</td>
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<td>23.1</td>
<td>23.1</td>
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<tr>
<td>ZAP-70 positive (n=15)</td>
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<td>26.7</td>
<td>26.7</td>
<td>33.3</td>
<td>11.3</td>
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<tr>
<td>Unfavorable cytogenetics (n=22)</td>
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<td>22.7</td>
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<tr>
<td>Age ≥ 65 years (n=30)</td>
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<td>Age ≥70 years (n=15)</td>
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<td>13.4</td>
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LOW-DOSE SUBCUTANEOUS CAMPATH (CAM) IS HIGHLY EFFECTIVE AND SAFE IN ADVANCED AND CHEMOTHERAPY-REFRACTORY B-CLL: RESULTS OF A PROSPECTIVE AND MULTICENTER PHASE II STUDY


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Cam is proven effective in refractory B-CLL, including patients pre-treated with fludarabine-based chemotherapy. Subcutaneous administration markedly reduces the severity of therapy-related adverse events. The aim of this prospective, multicenter study is to confirm the efficacy and safety of low-dose Cam in the treatment of refractory CLL. Forty patients were enrolled (14 female/26 male, median age 68 yrs, range 48-83 yrs) from January 2003 until May 2006; 15 patients were Binet’s stage C, 24 stage B, and 1 progressive stage A; 66% had unfavorable cytogenetics (including 17p-, 11q-, 6q, trisomy 12, and complex karyotype); 45% were CD38 and 62% ZAP-70 positive. All patients were refractory to alkyating agents, 25 to fludarabine, and 13 also to rituximab. Cam was given subcutaneously at 10 mg 3 times a wk for a maximum of 18 wks (median cumulative dose 510 mg; range 120-543). The OR by NCI criteria was 55% (CR 27.5%, PR 27.5%); with 52.5% SD and 12.5% PD. The median time of treatment failure was 12 months (range 6-22). Cam was equally effective in patients with worst clinical and biological features like age over 75 yrs, Binet’s stage C, active autoimmune cytopneas, 17p deletion, Fludarabine and Rituximab refractoriness. (Table 1).

DIVERSE IN VITRO ALEMTUZUMAB INDUCED APOPTOSIS ON T AND B LYMPHOCYTES OF B-CLL PATIENTS


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Introduction. Alemtuzumab, a monoclonal anti-CD52 antibody, has shown high efficacy against lymphoproliferative disorders such as B-cell chronic lymphocytic leukemia (B-CLL). However, its use results in a profound immunosuppression with decrease of CD4 and CD8 positive T lymphocytes leading to increased susceptibility to infections. The aim of this study was to assess the in vitro cytotoxic effect of alemtuzumab on normal T and neoplastic B lymphocytes obtained from B-CLL patients. Methods. Peripheral blood mononuclear cells (PBMC) from 44 B-CLL patients (19 at diagnosis and 25 previously treated) were collected and individually treated in vitro with alemtuzumab (10 ug/mL) and autologous serum in the culture as a source of complement. Spontaneous and alemtuzumab-induced apoptosis were quantified in T (CD3+) and B (CD20+) lymphocytes after 3 hours using an annexin-V flow

Oral Communications
cytometry based multiparametric assay. Results. Alemtuzumab induced more apoptosis on B than T lymphocytes of CLL patients. Spontaneous apoptosis and cytotoxic activity on normal T cells and neoplastic B cells were comparable in early vs advanced stage of disease (0-I Rai vs II-III-IV Rai). On the contrary, the activity of alemtuzumab on neoplastic B cells varied according to previous treatment. In untreated patients alemtuzumab induced apoptosis in 62.6% of neoplastic B cells vs 44.6% of previously treated patients (p=0.027). Although preliminary data indicated a minor spontaneous- and Alemtuzumab-induced apoptosis in T cells from untreated vs treated patients, this finding has not been confirmed by the final analysis. No difference were also found in treated patients when we compared therapeutic approaches with or without fludarabine. Conclusions. Our in vitro results showed that alemtuzumab is active against B-CLL cells in all stage of disease. However, in previously untreated patients, neoplastic B cells are more susceptible to alemtuzumab-induced apoptosis, with a major depletion of normal T cells. This observation supports the hypothesis that an earlier usage of Alemtuzumab in the treatment of B-CLL might result in a higher efficacy without increasing susceptibility to infections.

CO-045
CLINICO-PROGNOSTIC RELEVANCE OF SERUM AND CELLULAR EXPRESSION OF ADIPONECTIN IN EARLY B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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We analyzed the correlation between well-established biological parameters of prognostic relevance in B-cell chronic lymphocytic leukemia [ CLL] i.e., mutational status of the immunoglobulin heavy chain variable region [ IgVH], ZAP-70 and CD38-expression and adiponectin serum concentration in a cohort of 69 previously untreated Binet stage A B-cell CLL patients. The relationship among clinical, biological and bio-pathological parameters was analyzed by the multiple correspondence analysis (MCA). This analysis identified a subset of patients with a stable pattern of disease as defined by increased adiponectin serum levels (i.e., >.588 µg/mL), higher platelet count (>174x10^9/L), lower beta2-microglobulin [beta2-m] (<2.35 mg/L), presence of mutation in the IgVH and low percentage of either CD38-positive (<20%) or ZAP-70-positive (<20%) B-CLL cells. The univariate Cox proportional hazard model demonstrated that in addition with lower serum levels of adiponectin (p=0.01), the absence of IgVH mutational status (p=0.002) and ZAP-70 positivity (p=0.02) were associated with a shorter time to first treatment (TFT). However, in multivariate analysis only ZAP-70 positivity emerged as predictor of the TFT (p=0.008). Looking for cellular source of adiponectin we investigated the presence of adiponectin in B-CLL at gene expression level. In 60 B-CLL patients belonging to an independent series and heterogeneous with respect to the Binet stage. Such an analysis revealed low normalized expression values of adiponectin gene transcript indicating a reduced adiponectin circulating levels. In contrast, both adiponectin receptor 1 (AdipoR1) and AdipoR2 were highly expressed although a certain degree of variability among patients could be observed. Our results indicate that in early B-cell CLL clinico-biological profile including among other parameters adiponectin may provide a useful insight into the complex interrelationship of prognostic variables and simplify their interpretation. However, adiponectin may not replace the need for the determination of ZAP-70 and IgVH mutational status. The presence of AdipoR1 and AdipoR2 mRNA expression detected using RT-PCR suggest that the effect of adiponectin on cell proliferation was most likely specific and adiponectin receptor-mediated.

Figure 1. Duration of response in consolidated vs unconsolidated pts.
Chronic lymphocytic leukemia (CLL) B cells are not immortal and require signals from the microenvironment to maintain viability. Increasing evidence shows that neovascularization and angiogenic cytokines play a role in the biology of CLL. We measured Angiopoietin-2 (Ang-2) expression levels both in circulating leukemic cells and in bone marrow (BM) infiltrated tissue. Moreover, immunohistochemical staining with antibody against CD34, an endothelial cells marker, was performed to evaluate BM angiogenesis. We used ELISA assays to determine plasmatic concentrations of Ang-2 both in CLL patients and in normal controls. The results showed that CLL B cells consistently expressed mRNA for Ang-2. High expression of Ang-2, as measured at diagnosis, is associated with more advanced clinical stage, with unmutated immunoglobulin status and with unfavourable cytogenetics. Patients whose leukemic cells expressed Ang-2 had a shorter progression-free survival (median, 21 vs. 146 months; \( p = 0.002 \)). Increased CD38 expression on the surface of B-CLL cells has been shown to be an important prognostic factor. CLL with percentage of CD38+ cells over 30% showed a ten-fold higher median Ang-2 expression than CLL with CD38<30% (median, 4.58 vs. 0.49, respectively; \( p = 0.012 \)). Furthermore, CLL patients showed increased levels of soluble Ang-2 protein relative to normal controls (2419 vs. 1235 pg/mL, respectively). Of note, CLL patients with Ig-unmutated genes and poor prognosis had about 2-fold higher concentration of plasmatic Ang-2 than patients with Ig-mutated genes and good prognosis. As regards the BM microenvironment, both mean microvessel density (MVD) and hotspot density were significantly higher in B-CLL expressing high level of Ang-2 than in CLL subset with low (Ang-2) expression (MVD, 30.6 vs. 20.9; hotspot, 44.1 vs. 30.4, respectively, \( p = 0.01 \)). These results indicate a causal relationship between risk of progression, BM angiogenesis and Ang-2 expression in B-CLL.
**CHRONIC MYELOPROLIFERATIVE SYNDROMES**

**CO-049**

**AML1/MDS1/EVI1 INDUCES ESSENTIAL THROMBOCYTHYEMIA IN MICE INDEPENDENTLY OF JAK2 MUTATIONS**

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The molecular etiology of subgroups of essential thrombocythemia (ET) as well as some other myeloproliferative disorders (MPDs) has been recently better understood by the identification of an activating somatic point mutation (Valine 617 to Phenylalanine) in JAK2 detected in patients with MPDs. Approximately 25-57% of ET patients harbor this mutation, which is thought to be one of the dominant factors contributing to the disease. However, the pathways disrupted in at least half of ET patients who have wild type JAK2 are not known. The fusion gene AML1/MDS1/EVI1 (AME), a product of the t(3;21)(q26;q22) translocation, is associated with several hematopoietic disorders including t-MDS/AML, CML, CMMI, and ET. We have investigated the role of AME in these diseases by generating C57BL/6 mice that express this fusion oncogene in their bone marrow after bone marrow infection and transplantation. After a latency of 12±8 months, all the reconstituted mice invariably developed an ET-like disease. The disease was fatal and the animals displayed different phenotypes. Until 1-2 weeks before death, at which time about half of the mice were severely cytopenic, the peripheral blood profiles of the reconstituted AME mice were not significantly abnormal. The only striking difference was the platelet counts, which were consistently above the normal range (respectively 1456-3153 and 940-1608 K/µL for AME and control mice, p=0.00029). The platelets appeared dysplastic with anisocytosis, various degrees of degranulation, and giant size. Aggregation studies indicate that the platelet functions are impaired. The bone core biopsies of AME mice were especially remarkable for large and giant megakaryocytes with a tendency for clustering. The touch preparations of the biopsies showed a significant pro-apoptotic effect from 10 nM concentration (48% vs 8% in the control). On the other hand, at lower concentration (5 mM), Bortezomib blocked cell cycle in the G2 phase. Finally, this compound was able to down-regulate WT1 expression. No significant effects on cell differentiation were found. Because a spontaneous NF-kB activation has been reported in megakaryocytes from patients affected by myeloproliferative disorders, and WT1 gene has been reported to be over-expressed in chronic myeloproliferative diseases, bortezomib would be an attractive therapeutic tool for these malignancies, including essential thrombocythemia (ET) or idiopathic myelofibrosis (IMF). On these bases, we decided to investigate the possible efficacy of bortezomib in IMF: fragments of bone marrow biopsies from 15 patients affected by idiopathic myelofibrosis were cultured for 14 days, with or without the addition of PS-341. Cellular density was evaluated by computerized image analysis. In addition, bone marrow biopsies were graded for the presence of reticulin/collagen fibers and osteosclerosis (grade 0-3) and for the microvessel density by immunohistochemical staining for CD34 (grade 1-4). Transforming growth factor-β1 (TGF-β1) concentration was immunologically assayed in the supernatant of cultured bone marrow biopsies. Finally, megakaryocyte colony forming unit (CFU-MK) assay was performed on bone marrow samples of IMF patients, with or without PS-341. The median age of the patients was 64 years (59-75), 67% were male; 27% of them showed an intermediate Dupriez risk score and the median value of CD34+ cells were 0.4%. All except two cases showed a good bone marrow cellularity. In 3 cases IMF was preceded by ET diagnosis; in one case by that of polycythemia vera. Three patients showed chromosomal abnormalities: del(13q), t(4;14), t(1;17). Microscopic analysis revealed a decrease of the microvessel grade in 6/15 patients (40%) after PS-341 treatment. No significant differences were observed in the grading of the fibrotic area on the bone marrow cellularity. Nevertheless, bortezomib significantly reduced the CFU-MK formation, in particular of the large colonies, in all tested cases. On the other hand, PS-341 did not induce a significant reduction of TGF-β1 levels. These results appear particularly relevant in order to employ bortezomib in the treatment of IMF and in vivo trials would be useful for confirming the anti-proliferative activity exerted by bortezomib on megakaryocytic precursors.

**CO-051**

**IMATINIB MESYLATE INDUCES COMPLETE AND DURABLE RESPONSES IN ALL PATIENTS WITH THE FIP1L1-PDGFRα, POSITIVE HYPEREOSINOPHILIC SYNDROME. RESULTS OF A MULTICENTER PROSPECTIVE STUDY**

Rondoni D,1 Cilloni D,1 Paolini S,1 Ottaviani E,1 Messa F,1 Picculiga PP,1 Merante S,1 Buzzicano F,1 Tiribelli M,1 DeVivo A,1 Gottardi E,1 Vigna E,1 Messa F,1 Resti G,1 Giugliano E,1 Pane F,1 Saglio G,1 Zaccaria A,1 Baccarani M,1 Martinelli G1

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**Background and objectives.** The hypereosinophilic syndrome (HES) may be associated with the fusion of the PDGFRα gene with the FIP1L1 gene in chromosome 4 coding for a constitutively activated tyrosine kinase. This condition understand a clonal chronic myeloproliferative disorder and it is usually referred to as chronic eosinophilic leukemia (CEL). These cases of FIP1L1-PDGFRα rearranged CEL have been reported to be very sensitive to the TK inhibitor Imatinib mesylate (IM). Design and methods. A prospective multicenter study of the HES was established in 2001. 72 patients were treated with IM 100 to 400 mg daily. The obser-

**CO-050**

**PS-341 (BORTEZOMIB) INHIBITS BOTH PROLIFERATION OF MEGAKARYOBLASTIC CELLS IN VITRO AND MK COLONIES PRODUCTION IN PATIENTS AFFECTED BY IDIOPATHIC MYELOFIBROSIS**

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Department of Oncology, Transplant and Advances in Medicine, Section of Hematology, University of Pisa; 2Pathology II Unit, AOUP, Pisa, Italy

PS-341 (Bortezomib) is a dipeptide boronic acid proteasome inhibitor with antitumor activity that induces apoptosis in different human cancer cell lines. We investigated effects of PS-341 (Bortezomib) on cell proliferation, ROS production, cell cycle progression, induction of apoptosis and differentiation in a megakaryoblastic (M07-e) cell line. PS-341 was able to retain NF-kB in the cytoplasm and inhibit cell growth (IC50=20nM), in a dose/time-dependent way. This anti-proliferative activity resulted to be lineage-specific, because other leukemic cell lines (KG1a, K562/R7, HL60, HL60/DNR) were unaffected by the PS-341 treatment. Moreover, PS-341 in M07-e also increased ROS production and induced a significant pro-apoptotic effect from 10 nM concentration (48% vs 8% in the control). On the other hand, at lower concentration (5 mM), Bortezomib blocked cell cycle in the G2 phase. Finally, this compound was able to down-regulate WT1 expression. No significant effects on cell differentiation were found. Because a spontaneous NF-kB activation has been reported in megakaryocytes from patients affected by chronic myeloproliferative syndromes, and WT1 gene has been reported to be over-expressed in chronic myeloproliferative diseases, bortezomib would be an attractive therapeutic tool for these malignancies, including essential thrombocythemia (ET) or idiopathic myelofibrosis (IMF). On these bases, we decided to investigate the possible efficacy of bortezomib in IMF: fragments of bone marrow biopsies from 15 patients affected by idiopathic myelofibrosis were cultured for 14 days, with or without the addition of PS-341. Cellular density was evaluated by computerized image analysis. In addition, bone marrow biopsies were graded for the presence of reticulin/collagen fibers and osteosclerosis (grade 0-3) and for the microvessel density by immunohistochemical staining for CD34 (grade 1-4). Transforming growth factor-β1 (TGF-β1) concentration was immunologically assayed in the supernatant of cultured bone marrow biopsies. Finally, megakaryocyte colony forming unit (CFU-MK) assay was performed on bone marrow samples of IMF patients, with or without PS-341. The median age of the patients was 64 years (59-75), 67% were male; 27% of them showed an intermediate Dupriez risk score and the median value of CD34+ cells were 0.4%. All except two cases showed a good bone marrow cellularity. In 3 cases IMF was preceded by ET diagnosis; in one case by that of polycythemia vera. Three patients showed chromosomal abnormalities: del(13q), t(4;14), t(1;17). Microscopic analysis revealed a decrease of the microvessel grade in 6/15 patients (40%) after PS-341 treatment. No significant differences were observed in the grading of the fibrotic area on the bone marrow cellularity. Nevertheless, bortezomib significantly reduced the CFU-MK formation, in particular of the large colonies, in all tested cases. On the other hand, PS-341 did not induce a significant reduction of TGF-β1 levels. These results appear particularly relevant in order to employ bortezomib in the treatment of IMF and in vivo trials would be useful for confirming the anti-proliferative activity exerted by bortezomib on megakaryocytic precursors.
CO-052
MOLECULAR PROFILE OF CD34+ STEM CELLS IN ESSENTIAL THROMBOCYTHEMIA ACCORDING TO JAK2 EXPRESSION
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Essential Thrombocythemia (ET) is a Ph-negative chronic myeloproliferative disorder primarily characterized by abnormal proliferation of a malignant megakaryocytic clone and persistent thrombocytosis. The JAK2 V617F mutation has been reported in a variable proportion of ET patients (25 to 72% depending on the methodological approach). JAK2 mutation status divides ET patients into two distinct subgroups, with V617F-positive patients exhibiting many laboratory and clinical similarities to polycythemia vera. V617F-positive ET patients show higher levels of hemoglobin and white cells, hypercellular bone marrow (BM), increased risk of venous thrombosis and transformation to polycythemia vera, and greater sensitivity to hydroxyurea. Little is known about specific molecular abnormalities of the stem cell compartment in ET and, specifically, no data are available on the molecular characterization of JAK2 positive and JAK2 negative patients. Therefore, in the present study we utilized microarray technology to study BM CD34+ stem cells of ET patients, using Affymetrix HG-U133A GeneChip array, representative of 22,283 transcripts. Gene expression profiles of CD34+ cells were performed in patients with JAK2-negative and JAK2-positive ET to identify differentially expressed genes. Eight V617F-positive (5 men and 3 women; median age 66 years; range 26-65 years) and 8 V617F-negative patients (2 men and 6 women; 49 median age; range 30-65 years) with ET diagnosed according to Polycythemia Vera Study Group criteria were studied. The median platelet count was 912×10^9/L for V617F-positive and 834×10^9/L for V617F-negative patients. The patients were either newly diagnosed or off cytotoxic treatment for at least 5 months. CD34+ cells were isolated using immunomagnetic cell sorting. The JAK2 V617F mutation was identified by RT-PCR followed by enzymatic digestion with BsaXI restriction enzyme. Different functional categories and JAK2-dependent genes were analyzed. Overall, we did not find any significant difference in the gene expression profiling between the V617F-positive and V617F-negative ET patients. Keeping in mind these results, we can hypothesize that post-transcriptional events are responsible of the clinical and laboratory findings which characterize the two subgroups.

CO-053
SYSTEMIC MASTOCYTOSIS: A GIMENA MULTICENTRIC SURVEY
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Introduction. To evaluate clinical and pathological features, treatments and outcome of patients (pts) with Systemic Mastocytosis (SM).

Methods. A retrospective study, conducted over 1995-2006, in pts admitted in 16 Italian hematology divisions in tertiary cares or university hospitals, in whom SM was diagnosed. Results. 36 valuable cases of SM were collected (median age 62 y.o.; M/F 20/16) and classified according to the WHO criteria: Mast Cell Leukemia in 13 pts, Aggressive SM in 15 and Indolent SM in 3; the remaining 5 had SM with associated clonal non-mast cell-lineage hematologic disease. Skin was the principal extramedullary organ involved by uncontrolled proliferations of mast-cells (24 pts) followed by spleen (17), liver (16), and cardiovascular system (13). Molecular biology studies were performed in 24 pts: 16 showed the c-kit point mutation D816V, in another patient a different c-kit mutation was found while in 4 pts additional gene defects and karyotype abnormalities were recognized. Treatments were very heterogeneous, and the same patient could have received different therapies after failure of the previous one. Imatinib (400 mg/day) was used in 17 pts (11 as first line therapy, 5 and 1 as second and third line respectively); 6 Interferon (5x3 MU s.c. weekly) was employed in 9 patients (6 as first line therapy, 2 as second and 1 as third line); 2-CD (0.14 mg/kg) was administered in 8 pts (1 as first, 1 as second and 1 as third line therapy); 2 patients underwent HSCT as second and third line respectively. The overall response rate to Imatinib, the most frequently employed drugs, was of 35%, registering 1 complete remission and 4 partial remission. All but one responsive patients did not present c-kit point mutation D816V. Two pts were lost to the last follow-up; among the remaining 34 patients, 5 (8%) died for progression of SM; two patients in CR of disease died respectively from cardiac and acute myeloid infarction. The actuarial Kaplan-Meier curve at 10 years showed an overall-survival of 85%. Conclusions. SM is a rare disease, characterized by a severe and life-threatening mediator-related symptoms but, on the other hand, with a low mortality rate. D816V c-kit mutation is frequent and associated with resistance against Imatinib. In fact only 1 patient showed a CR. Because of the rarity of this disease, an effective standard care is lacking. More data are needed to find new and successful therapeutic strategies; it is possible that new tyrosine kinase inhibitors could allow to achieve clinical and molecular remission of disease, crossing resistance to Imatinib due to c-kit mutation, in order to improve above all quoad valitudinem prognosis of these pts.

Table. Data about response to treatment.

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CR: complete remission; PR: partial remission; UR: unresponsive.

CO-054
ANTI-IL-5 RECOMBINANT HUMANIZED MONOCLONAL ANTIBODY (MEPOLIZUMAB) FOR THE TREATMENT OF IDIOPATHIC HYPEREOSINOPHILIC SYNDROME (HES)
Istituto di Ematologia ed Oncologia Medica L. e A. Seràgnoli, University of Bologna, Italy

Treatment of HES includes corticosteroids, chemotherapeutic agents, interferon-alpha, and, more recently, imatinib mesylate for the myeloproliferative forms with the involvement of PDGFRα. IL-5 is a cytokine involved in regulating several aspects of eosinophils production, activation, and tissue recruitment. Mepolizumab is a recombinant humanized monoclonal antibody against IL-5. It's role in HES has to be established. We treated four patients, negative for FIP1L1-PDGFRα fusion transcript, with intravenous high dose (750 mg) of Mepolizumab, according to clinical trials (Glaxo, MHE 185, patient 1, 2 and 3) or by therapeutic use (pt 4). Patient 1 was a 19-year old woman who on diagnosis showed generalized muscle rigidity and pain even with eosinophils count of 20,000/mm³. She was treated with oral methyl-prednisolone and then with imatinib, but even though she had no relapse of muscle symp-
toms, she maintained high peripheral blood eosinophilia. She was subsequently treated with two doses of Mepolizumab. She was resistant also to this therapy. Patient 2 was a 63-year old man with a two year history of idiopathic HES primarily involving lung and with recurrent asthma symptoms. Treatment with prednisone and imatinib made him stable for ten months. At the time of relapse he started Mepolizumab therapy and his eosinophils were already < 200/mmc for nine months of the study, than and he had an increase six months after from the last dose. Patient 3 was a 65 year old man with a long history of idiopathic HES with instrumental pulmonary involvement stabilized with prednisone. He also suffered iatrogenic insulin-dependent hyperglycemia. When he started Mepolizumab administration prednisone was reduced and progressively suspended. Until now he maintained stable eosinophil count with a 3-months administration. Patient 4 was a 25-year old woman with sinusitis/nasal polyposis and respiratory difficulty. She had been treated with corticosteroid but she had frequently recurrence of her symptoms also in absence of peripheral eosinophilia. She had a prompt resolution of HES symptoms after Mepolizumab infusion. No one had adverse event during and after the infusion. Acknowledgments. COFIN 2003 (Molecular therapy of Ph+ leukemias), by FIRB 2001, by the University of Bologna (60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R.), by Fondazione Del Monte of Bologna and Ravenna (Italy) and A.I.L. grants, LeukemiaNet grants.

CO-055

EPH3A IS CONSTITUTIVELY ACTIVATED IN CHRONIC MYELOBLASTIC LEUKEMIA AND CAN BE TARGETED BY DASATINIB OR BY MONOCLONAL ANTIBODIES


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Eph receptors tyrosine kinase are involved in many key development processes. Although Ephs receptors are undetectable in adult tissues, they are overexpressed in many tumors, suggesting a possible role of these PTKs in oncogenesis. Activation of tyrosine kinases is a common finding in the pathogenesis of chronic myeloproliferative disorders (CMPD) and many clinical trials with new TK inhibitors are ongoing. Dasatinib (Bristol-Myers Squibb) exhibits an interesting inhibitory activity on many PTKs. The aim of this study was to investigate the role of EphA3 in CMPD and to explore the possibility to target EphA3 with TK inhibitors or monoclonal antibodies. EphA3 mRNA was analyzed using Real Time PCR in 334 samples obtained from 280 CMPD patients (280 BM and 54 PB) and in 38 healthy controls (18 PB and 20 BM). 73 were PV, 65 ET, 24 IM, 24 CMMML, 8 HES, 50 CML and 86 Ph-CML. In 10 patients and 10 healthy subjects CD34+ cells were selected and analyzed by FACS. The expression of EphA3 protein and its localization were examined using Western Blot and immunofluorescence analysis. The effects of EphA3 overexpression were studied by transfecting EphA3 plasmid in 293T e COS cells negative for EphA3 expression. In addition, sequencing of TK domain was performed in 48 EphA3+ patients. Finally, samples were incubated with Dasatinib 20nM for 6 hrs and evaluated for cell proliferation by incorporation of 3H thymidine and MTT assay. EphA3 expression was analyzed by FACS (Annexin V) and colony growth by methylcellulose culture. Normal BM, PB and CD34+ cells are negative for EphA3 expression (mean value of ∆Ct=19) By contrast EphA3 was found significantly increased in 45% of PV (mean value of ∆Ct=11) in 55% of ET (mean value ∆Ct= 10,5) in 90% of CMMML mean value ∆Ct=5), in 100% of IM (mean value =4), in 30% of CML (mean value of δCt=9), 15% of HES (mean value of ∆Ct=12) and 80% of Ph-CML (mean value of ∆Ct=9). CD34+ cells presented significantly higher levels as compared to the corresponding unfractioned sample (p=0,001). Western Blot and immunofluorescence confirmed the presence of EphA3 protein in EphA3 overexpressing cells and revealed abnormal phosphorylation of the receptor. Dasatinib incubation induced a significant inhibition of EphA3 phosphorylation. Moreover, Dasatinib induced significant apoptosis (mean value 32±12%), colony growth reduction (mean value of 34,2 vs 76,5) and proliferation rate inhibition (48%) in EphA3+ cells compared to normal controls and to EphA3 negative cells in which we were unable to observed any significant effect. Similar effects were observed after incubation with a specific antibody blocking the receptor. No kinase domain mutations were found in EphA3 overexpressing cells. Conclusions EphA3 is abnormally expressed in different hematological malignancies with a significant overexpression in CMPD as compared to normal controls. The inhibition of EphA3 phosphorylation induced by Dasatinib or by the antibody results in growth arrest and apoptosis of EphA3 overexpressing cells. Therefore, EphA3 may represent a potential candidate for a molecular therapy in chronic myeloproliferative disorders.

CO-056

FAVORABLE OUTCOME OF PREGNANCY IN ESSENTIAL THROMBOCYTHEMIA PATIENTS TREATED WITH INTERFERON ALPHA. A REPORT FROM THE REGISTRO ITALIANO TRONOCITEMIA (RIT)

Melillo L1, Tieghi A1, Valente D1, Comitini G2, Cardoni A4, Ciancia R4, Martinelli V1, Radaelli E1, Latagliata R3, Specchia G4, Scalfuzi PR4, Marino A4, Palmieri E4, Cedrone M5, Cascavilla N1, Gugliotta L1 on behalf of the Registro Italiano Trombocitemia (RIT)

1Haematology Unit S. Giovanni Rotondo; 2Haematology Unit Reggio Emilia; 3Haematology Unit Udine; 4Haematology University Napoli; 5Haematology Unit Milano; 6Haematology University Roma 1; 7Haematology University Barì; 8Haematology Unit Reggio Calabria; 9Haematology Unit Avellino; 10Haematology Unit S. Giovanni, Italy

Essential Thrombocythaemia (ET) is diagnosed in the childbearing age in about 20% of patients. Fertility reduction and adverse outcomes of pregnancy due to thrombotic or haemorrhagic complications represent several challenges in the management of ET patients. One hundred and eighteen pregnancies occurring in 91 women with ET, observed in 17 Italian Haematological Centers from 1998 to 2006 and registered in the RIT are object of this retrospective study. The patients, diagnosed according to the PVSG or WHO criteria, had a median age at conception of 31 years (21-45). Median platelet count was at diagnosis 996 ×10^9/Liter (range 489-2140) and at delivery 520×10^9/Liter (232-1530). A cytoreductive treatment at conception was registered in 36 pregnancies (Interferon-alpha 20, Anagrelide 9, Hydroxyurea 6, and Busulphan 1). Beside 81 (69%) live births, 24 (20%) spontaneous abortions (17 in the first trimester, 7 in the second trimester), 4 (3%) still births, and 5 (4%) voluntary abortions were described. Four pregnancies are ongoing. Of the 81 live births 12 (15%) were premature births and 67 of the remaining 69 pregnancies were associated with a normal foetal growth. Three pregnancies in patients with antiphospholipid antibodies resulted no complicated. Two cases of pre-eclampsia were also observed. The delivery was by caesarean section in 42% of cases. In 85 (72%) pregnancies, aspirin treatment (mainly 100 milligrams/day) was reported, associated in 18 cases to prophylactic LMWH one week before delivery and for six weeks post-partum. Pregnancy was successful in 62.5% of patients treated with ASA, not significantly different as compared with 65.4% in the women not receiving ASA (p>0.91, Chi-square test). The Interferon alpha, eight cases of not to attempt pregnancy, was administered during 18 pregnancies considered at high thrombotic risk. Sixteen of these pregnancies were valuable (1 ongoing and 1 elective abortion) and, interestingly, all cases ended in live births. The outcome was significantly different as compared with patients not receiving Interferon alpha (p<0.01, Fisher’s exact test). These data confirm that fetal morbidity and mortality rate is not negligible in ET. Cytoreductive therapy with interferon alpha seems able to protect against fetal loss. The epidemiological, clinical and biological data on pregnancy in ET are now object of a prospective study by the RIT (GIMEMA project) in order to improve the care that pregnant ET women could receive under the joint care of haematologists and obstetricians.

haematologica/the hematology journal | 2007; 92(s3) | 27
**CO-057**

**LOW DOSE RITUXIMAB SHARES SIMILAR ACTIVITY BUT SLOWER TIMING OF RESPONSE THAN STANDARD DOSE IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA**


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Introduction. Rituximab 375 mg/sqm weekly for 4 weeks has significant activity in patients with immune thrombocytoppenia (ITP). In this setting, different biological and clinical evidence suggests the possible use of lower doses of Rituximab. Methods. Twenty-one adult patients, median age 44 years (range 16-71 years), with previously treated and symptomatic ITP (18 idiopathic, 3 secondary) were enrolled in the registry as those satisfied the inclusion criteria and were enrolled in the registry as 1 (54%), 2 (40%) and 3 (6%). ITP diagnosis occurs in young adults (83%), mainly in women (57%). Mucosal bleeding (64%) is more frequent than hematomas or hemorrhoids (15%) but 73% of patients did not require transfusions. In the prospective study based on 814/1,234 (66%) cases of the registry (type 1=47%, 2=47%, 3=6%) 147/815 (18%) were treated in a year for 318 bleeding episodes and 87 minor or major surgeries. BSS >10 (hazard ratio = 6.8, 95%CI 3.8-12.3), bleeding time <20 min (BT = 5.3, 3.1-9.8), VWF:RCo <30 U/dL (3.2, 1.7-5.9) and VIII:C <20 U/dL (4.1, 2.4-7.0) were significantly associated with higher risk of bleeding. By multivariate model including all the variables, BSS <5.5, BT <8.1, VWF:RCo <30 U/dL and VIII:C <20 U/dL were the most significant determinant of bleeding. The bleeding-free survival at one year was significantly different in type 3 (52%) versus types 1 (96%) and 2 (91%) ITP. On the other hands, patients with WVECo >80 U/dL and VIII:C >40 U/dL showed always BSS <5 with the lowest incidence of bleeding (5.0±100 patient-years). A total of 292 DDAVP injections were used to manage bleeding within a group of patients with types 1 (65%) and 2 (35%) ITP. 49% of the injections of VWF concentrates were used to treat bleeding and surgeries in type 3 (75%), type 2 (34%) and type 1 (15%) ITP. Conclusion. Based on the results of this prospective study, we can confirm that BSS is an important clue to predict clinical bleeding and the need of therapy with DDAVP and VWF concentrates. In cases with WVECo >80 U/dL and VIII:C >40 U/dL bleedings occurs very rarely in agreement with their respectively low BSS.

**CO-058**

**INCIDENCE AND DETERMINANTS OF BLEEDING IN VON WILLEBRAND DISEASE: RESULTS OF THE FIRST PROSPECTIVE MULTICENTER STUDY ON 814 ITALIAN PATIENTS**

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Background. von Willebrand disease (VWD) is the most frequent inherited bleeding disorder and is due to quantitative and/or qualitative defects of von Willebrand factor (VWF). Despite its improved knowledge among hematologists, no data on the incidence and determinants of bleedings requiring specific treatments are available until now. Aims and design of the study. To determine the incidence and determinants of bleedings requiring therapy with DDAVP and/or VWF concentrates in VWD, a national registry was organized by using a database devised to collect detailed retrospective information. Patients included in the registry were followed up for one year and prospective data on number, type and management of bleeding episodes was analyzed. Methods. All patients were diagnosed following recommendations of the ISTH-SSC on VWF with bleeding severity score (BSS) calculated at enrollment. Diagnoses of VWD were confirmed by the coordinating center using multiparametric analysis in plasma and mutations of VWF gene in types 2 and 3. For different risk categories the incidence of bleeding (mucosal and non-mucosal bleeding) was calculated. Bleeding-free survival was computed with the Kaplan-Meier method and a Cox’s proportional hazard model was used to calculate the risk of bleeding in different risk categories. (hazard ratio = HR). Results. In the retrospective study, 1,234/1,529 (81%) cases satisfied the inclusion criteria and were enrolled in the registry as types 1 (54%), 2 (40%) and 3 (6%). VWD diagnosis occurs in young adults (83%), mainly in women (57%). Mucosal bleeding (64%) is more frequent than hematomas or hemorrhoids (15%) but 73% of patients did not require transfusions. In the prospective study based on 814/1,234 (66%) cases of the registry (type 1=47%, 2=47%, 3=6%) 147/815 (18%) were treated in a year for 318 bleeding episodes and 87 minor or major surgeries. BSS >10 (hazard ratio = 6.8, 95%CI 3.8-12.3), bleeding time <20 min (BT = 5.3, 3.1-9.8), VWF:RCo <30 U/dL (3.2, 1.7-5.9) and VIII:C <20 U/dL (4.1, 2.4-7.0) were significantly associated with higher risk of bleeding. By multivariate model including all the variables, BSS <5.5, BT <8.1, VWF:RCo <30 U/dL and VIII:C <20 U/dL were the most significant determinant of bleeding. The bleeding-free survival at one year was significantly different in type 3 (52%) versus types 1 (96%) and 2 (91%) ITP. On the other hands, patients with WVECo >80 U/dL and VIII:C >40 U/dL showed always BSS <5 with the lowest incidence of bleeding (5.0±100 patient-years). A total of 292 DDAVP injections were used to manage bleeding within a group of patients with types 1 (65%) and 2 (35%) ITP. 49% of the injections of VWF concentrates were used to treat bleeding and surgeries in type 3 (75%), type 2 (34%) and type 1 (15%) ITP. Conclusion. Based on the results of this prospective study, we can confirm that BSS is an important clue to predict clinical bleeding and the need of therapy with DDAVP and VWF concentrates. In cases with WVECo >80 U/dL and VIII:C >40 U/dL bleedings occurs very rarely in agreement with their respectively low BSS.
were smaller in size in comparison to the controls. The DNA content measured after 96 hours, pointed out a significant lower (4n) level of polyplody in the AME positive cells compared with the controls (8n). We also checked in the K562 cell line by semi-quantitative PCR the expression of several regulatory genes implicated in megakaryopoiesis. Before PMA incubation, AME positive cells showed a slight up-regulation of GATA1, cMPL, PF4 and BCL2, while NFE2 and BCL-XL were slightly down-regulated. Interestingly, after PMA stimulation, except GATA1 and BCL-XL that were up-regulated, all the others were strongly down-regulated. It seems that AME interferes with the megakaryocytic program, causing impairment in MKs differentiation with arrest in polypliodization, increased apoptosis and overproduction of immature platelets. The molecular mechanism appears complex, affecting concurrently transcription factors essential for MKs differentiation, apoptosis regulatory genes and genes implicated in platelets production.

**CO-060**

**MOLECULAR CHARACTERIZATION OF FIVE FAMILIES WITH INHERITED SEVERE FXIII DEFICIENCY**

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Factor XIII (FXIII) deficiency is a very rare (1:2,000,000) severe autosomal recessive bleeding disorder, mostly due to mutations in the coagulation FXIII A subunit gene. Only a few patients have been genetically characterized so far. The HGMD web site reports 65 different mutations already published. We have studied the molecular basis of FXIII deficiency in FXIII deficient patients from five unrelated Italian families. The coding region, intron-exon boundaries and 5’ and 3’ untranslated regions of the FXIII gene encoding the A subunit were amplified and sequenced. Candidate mutations were identified in all the patients. Three novel mutations occurred in three patients. These include a novel homozygous deletion of two base pairs (bp) in exon 14 (2103-2104 Del CT, frameshift from Leu667).

<table>
<thead>
<tr>
<th>P2</th>
<th>SEX</th>
<th>AGE</th>
<th>BLEEDING SCORE</th>
<th>FXI ACTIVITY</th>
<th>DNA MUTATIONS</th>
<th>DOMAIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M</td>
<td>33</td>
<td>1</td>
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<td>AAPA4.903</td>
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<tr>
<td>B</td>
<td>F</td>
<td>52</td>
<td>3</td>
<td>2%</td>
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<td>GCC102.T835</td>
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<tr>
<td>C</td>
<td>M</td>
<td>60</td>
<td>2</td>
<td>0.9%</td>
<td>Gln150X hetero</td>
<td>GAA104.n600</td>
</tr>
<tr>
<td>D</td>
<td>F</td>
<td>18</td>
<td>4</td>
<td>0.4%</td>
<td>Gly117X hetero</td>
<td>GAA104.A403</td>
</tr>
<tr>
<td>E</td>
<td>M</td>
<td>33</td>
<td>0</td>
<td>0.5%</td>
<td>Gln150X hetero</td>
<td>GAA104.A403</td>
</tr>
<tr>
<td>F</td>
<td>F</td>
<td>35</td>
<td>2</td>
<td>1%</td>
<td>Cys309X hetero</td>
<td>GAA104.n681</td>
</tr>
</tbody>
</table>

This deletion causes a frameshift from Leu667 and the formation of a stop codon at aminoacid position 681. The second patient is compound heterozygote for two missense mutations, a previously reported one (Arg260His), and a novel transition in exon 4 (nt.661 CCT>CTT) predicting a Pro163Leu in the core domain. The third patient presents a novel homozygous nt.2227 AGC>AAG transition in exon 15, predicting a Ser708Asn in Barrel 2 domain. The remaining two patients have two previously reported mutations: a 4 bp homozygous deletion in exon 11 (nt.1489-92 Del ATT), previously reported to occur in the Vicenza Area, and a homozygous non-sense mutation in exon 8 nt.1080 CGA>TGA predicting an Arg326X in the core domain. All the mutations were not detected in 110 normal alleles. The novel mutations are considered to be disease-causative since they occurred at aminoacid residues highly conserved among different species (pig, monkey, mouse, dog). Molecular modelling will help in elucidating the structural role of these mutations.

**CO-061**

**RITUXIMAB IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)**

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Background. Rituximab, a chimeric anti-CD20 monoclonal antibody effective in B-cell depletion, may be useful in autoimmune disorders by interfering with the production of auto-antibodies. Aims. To investigate the efficacy of Rituximab in patients with resistant ITP. Patients and Methods. Nineteen adult ITP patients (5 males, 14 females; median age 43.2 years (21.1-67.6) were treated with Rituximab (375 mg/m²/weekly for four doses). Median time between diagnosis and start of Rituximab was 2.1 years (0.2-53.1). All patients had already received at least two lines of therapy (median 3; 2-6) prednisone, pulsed high-dose dexamethasone, azathioprine, immunoglobulins, interferon or splenectomy. At the start of Rituximab, median platelet count was 10x10^9/L (3.3-30x10^9/L). Response definitions: complete response (CR), platelet count ≥150x10^9/L; partial response (PR), >50-150x10^9/L; minimal response (MR), >20x50x10^9/L; no response (NR)≤20x10^9/L. After completing therapy, patients were evaluated for platelet count after 1 and 3 months, and thereafter every 3 months until relapse or start of a different treatment. Peripheral blood B lymphocytes were evaluated by flow-cytometry as CD19+ cells before treatment, 1 and 3 months after stopping therapy, and then every 3 months up to recovery. Results. One month after Rituximab therapy, 17/19 patients are valuable for response. Eight responses (2 CR, 5 PR, 1 MR; 47%) and 9 NR (53%) were observed. Four relapses occurred 1, 5, 18 months respectively, after response. Median follow-up of all treated patients was 6.8 months (1.1-48.5). Median follow-up of all responsive patients was 8.1 months (5.3-18.7). Before starting therapy, 15/19 cases were valuable for flow-cytometry studies. The median baseline value of peripheral blood CD19+ B cells was 137x10^6/L (3-33x10^6/L). Before starting therapy, CD19+ cell numbers. No serious infections were observed during the clinical follow-up. No patient had to stop therapy because of severe side effects.

**CO-062**

**THE RISK OF PULMONARY EMBOLISM DUE TO PROXIMAL DEEP VENOUS THROMBOSIS OF THE LEGS DIFFERS IN PATIENTS WITH DIFFERENT KINDS OF INHERITED THROMBOPHILIA**

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Introduction. It is uncertain whether the presence of inherited thrombophilia influences the risk of developing pulmonary embolism (PE) and whether different thrombophilic alterations are associated with different incidences of PE. The present study is aimed to investigate the risk of PE among patients with deep vein thrombosis (DVT) and inherited thrombophilia. Patients and Methods. We studied 920 patients (M/F 397/523) with proximal DVT of the legs with or without PE referred for thrombophilia screening, after exclusion of patients with overt can-
Venous thromboembolism (VTE) is a major complication of multiple myeloma (MM) therapy recently observed with the increasing use of thalidomide in patients with newly diagnosed disease. The pathogenesis of thalidomide-induced VTE is not well recognized and data concerning the relationship between thrombophilic alterations and the risk of VTE are still limited and conflicting. Aim of the present study was to investigate such a relationship in 266 patients who received four months of therapy with thalidomide (thal, 200 mg/d) and pulsed high-dose dexamethasone (dex) in preparation for double autologous transplantation. The rate of VTE events in the whole group of patients was 11.6%. The risk of VTE was 26.3% (86.2% patient-years) among the first 19 patients who did not receive any prophylaxis against thrombosis and 10.6% (55.5% patient-years) among the time of DVT or PE. Three hundred and fifty-three patients (38.3%) had deficiency of antithrombin (AT, n=16), protein C (PC, n=25), protein S (PS, n=25), factor V Leiden (FVL, n=168), prothrombin G20210A (PT-GA, n=87), or multiple abnormalities (n=34), and 567 had no known defect. We analyzed the incidence of PE at the time of the first DVT in the patients with thrombophilia in comparison with those with no known defect. Results. First DVT was followed by PE in 242 patients (26%); the risk of PE was increased in patients with AT deficiency (relative risk, RR, 2.4, 95% CI 1.6-3.6) or with PT-GA (RR 1.5, 95% CI 1.1-2.0) and decreased in those with FVL (RR 0.7, 95% CI 0.5-1.0) in comparison with those with no known inherited defect. The increase in risk for PE associated with AT deficiency or PT-GA was more pronounced in women (RR 4.0, 95% CI 2.4-6.5 and 1.8, 95% CI 1.1-2.7, respectively) and in patients younger than 45 years (RR 3.6, 95% CI 2.3-5.6 and 2.0, 95% CI 1.3-3.0, respectively). In the subgroup of younger women the risk associated with AT deficiency was 4.9-fold increased (95% CI 2.6-9.2) and the risk associated with PT-GA was 2.3-fold increased (95% CI 1.3-3.9). Discussion. The design of our study is susceptible of underestimation of the rate of PE among DVT patients. However, there is no reason to think that the pattern of underestimation should have differed among the patient subgroups, having the diagnostic procedures been carried out before the referral to the Thrombosis Center. Therefore, the estimate of the relative risk between patients is quite reliable. Our data suggest that patients with DVT have different risks of PE according to the genotype and that AT deficiency or PT-GA can lead preferentially to PE. The risk associated with such abnormalities is further increased in women and in younger individuals.
Multiple myeloma (MM) is a clonal B-cell malignancy characterized by the accumulation of malignant plasma cells within the bone marrow (BM). Despite treatment with alkylating agents, anthracyclines, corticosteroids, and bortezomib as well as high-dose therapy and stem cell transplantation MM remains incurable due to both intrinsic and acquired drug resistance. Therefore, new therapeutic strategies are needed to improve patient outcome. Preliminary data from clinical studies indicate that Arsenic Trioxide (ATO) has clinical activity as a single agent in MM, and combination therapies are being investigated. We recently reported that PD184352 (PD) (Pfizer, Ann Arbor, MI), a highly selective inhibitor of MEK phosphorylation and activation, significantly enhances ATO-mediated apoptosis in acute myelogenous leukemia via multiple intrinsic apoptotic pathways activation (Lunghi et al. Blood 104: 519-525, 2004; Lunghi et al. Blood 107: 4549-4553, 2006). The aim of this study was to investigate whether the combined treatment with PD and ATO has cytotoxic effects on MM cells. We first analyzed the pharmacological interactions between PD (2 ÂM) and ATO (1-2 ÂM) using a fixed-ratio experimental design on 7 human myeloma cell lines (MM) with varying p53 status (RPMI 8226, U266, XG-1, XG-6, JN5, HS-SULTAN, NCI-H929) and found that the combined treatment resulted in the synergistic (Combination Index <1.0) induction of apoptosis in NCI-H929, XG-1, XG-6, RPMI 8226, SULTAN and JN5 HMCL. Conversely, the combination of PD plus ATO had a slight antagonistic effect on apoptosis induction in U266 HMCL (Combination Index >1). Moreover, PD plus ATO-induced cytotoxicity on HMCLs was maintained also in presence of the potent pro-survival anti-apoptotic protein Mcl-1 (50 ng/ml) in a co-culture system with bone marrow stromal cells. Similarly to HMCLs we found that the treatment with PD significantly enhanced the apoptosis of fresh purified MM cells induced by ATO (p<0.01) in 9 out of 12 patients of MM analyzed. Conversely, PD treatment partially attenuated (n=2) or did not affect (n=1) the ATO cytotoxicity in normal bone marrow 8 cells. To investigate the molecular mechanisms by which PD plus ATO induced MM MM cells apoptosis we first compared the effect on caspase activation in MM cells expressing wild type (wt) p53 or mutated p53. By Western immunoblotting analysis, we demonstrated the involvement of caspase-8, -9 and -3 in PD plus ATO-induced apoptosis in MM cells with mutated p53 and primarily caspase-9 and -3 in dual treated cells expressing wt p53. In addition, PD plus ATO induced a p53-dependent up regulation of Puma, Bax and Bak in HMCLs with wt p53, and p53-specific siRNA cells apoptosis (p<0.01) reduced the effect on caspase activation in MM cells treated with the caspase-8-mediated proteolytic activation of Bid, a key protein involved in the cross-talk between the intrinsic and extrinsic apoptotic pathways, closely correlated with the caspase-9 activation and loss of mitochondrial membrane potential observed in dual treated HMCLs with mutated p53. Finally, in the responsive HMCLs both with wt or mutated p53, the combined treatment increased the level of the pro-apoptotic Bim and decreased its neutralizing anti-apoptotic protein Mcl-1 (ATO-mediated) causing an imbalance between these proteins that positively influenced the pro-apoptotic efficacy of the combination. In conclusion, our data indicate that the disruption of MEK pathway potentiates the apoptotic effect of ATO in MM cells through the activation of both extrinsic and intrinsic (caspase-8 -Bid-mediated) pathways in HMCLs with mutated p55 or through the primarily activation of caspase-9 in cells with wt p53. Also the contribution of Bim pathway. These findings suggest that a strategy combining ATO with disruption of MEK pathway warrants attention in MM.

**CO-067**

**ASSOCIATION OF BORTEZOMIB, DOXORUBICIN AND DEXMETHASONE (PAD) IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS**

Palumbo A,1 Gay F,2 Falcone A,3 Pescotta N,3 Callea V,3 Caravita T,3 Morabito E,3 Falco P,3 Larocca A,3 Avonuto I,3 Musto P,3 Cascavilla N,3 Boccardo M1

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Introduction. Bortezomib has shown significant activity in patient with multiple myeloma (MM); it demonstrates synergy with doxorubicin and its efficacy is increased by the addiction of dexamethasone. In this study,
we assessed the safety and efficacy of the association of bortezomib, doxorubicin and dexamethasone (PAD) in patients with relapsed/refractory MM. Methods: Sixty-four relapsed/refractory patients received bortezomib (1.3 mg/m^2 i.v. days 1, 4, 8, 11), doxorubicin (20 mg/m^2 i.v. days 1, 4) or pegylated-liposomal-doxorubicin (30 mg/m^2 i.v. day 1) and dexamethasone (40 mg p.o. days 1-4) for up to six 28-days cycles. Results. Median time from diagnosis was 31 months and median number of prior therapies was 2. 57.8% of patients received prior autologous transplantation and 26.6% bortezomib based-regimen. Forty-three patients (67%) achieved partial response (PR) including 16 (25.0%) who achieved at least very good partial response (VGPR). Responses were equal or superior to that induced by previous treatment schedules in 68.8% of patients. The VGPR (17.6% versus 28.1%) and PR (41.2% versus 37.5%) rates were similar between patients who received prior bortezomib regimens and those who did not. The 1-year progression-free survival (PFS) was 34.5% in patients treated with PAD and 31.2% in the same patients after the previous line of therapy (hazard ratio (HR) 1.20; 95% CI 0.65-2.19, p=0.02). The 1-year PFS was not different between patients who received prior bortezomib based-regimens or not (15.8% versus 38.7%, HR 1.07, 95% CI 0.51-2.20, p=0.86). The 1-year PFS in patients who achieved VGPR or CR was 83.3% and 16.1% respectively. HR 1.79, 95% CI 0.83-3.88, p=0.043. The 1-year PFS among patients with beta2microglobulin levels lower or equal to 4.0 mg/dL was 32.2% and 41.0% in those with higher beta2microglobulin levels (HR 1.07, 95% CI 0.51-2.20, p=0.86). The 1-year PFS in patients who achieved VGPR or CR was 33.5% and 16.1% in those with higher beta2microglobulin levels (HR 1.07, 95% CI 0.51-2.20, p=0.86). The 1-year PFS in patients who achieved VGPR or CR was 33.5% and 16.1% in those with higher beta2microglobulin levels (HR 1.07, 95% CI 0.51-2.20, p=0.86).

**Discussion.** The PAD regimen showed a high proportion of responses with manageable and predictable toxicities. Both responses and PFS were not influenced by previous bortezomib treatments and serum beta2microglobulin levels.

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**CO-068**

**THE PROTEASES INHIBITOR BORTEZOMIB STIMULATES THE OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL CELLS IN VITRO AND MAY INCREASE OSTEOBLAST FORMATION IN VIVO IN MULTIPLE MYELOMA PATIENTS**

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The proteasome inhibitor Bortezomib may increase osteoelastic related markers in multiple myeloma (MM) patients, however its potential osteocastic stimulatory effect is not known. To clarify this issue in this study we checked the effect of Bortezomib either on osteoclastic differentiation and formation or on osteoblast proliferation, survival and function. In long-term human BM cultures we found that Bortezomib did not reduce the number of both early bone marrow (BM) osteoblast progenitors (CFU-F) and late ones Colony Forming Bone nodules (CFU-BF). Using the human osteoblast like cells MG-63 and immortalized normal osteoblasts (HOBIT) we found that Bortezomib at concentration ranging between 2nM and 5nM did not inhibit osteoblast proliferation or induce osteoblast apoptosis. On the other hand we found that Bortezomib significantly induced osteoblast phenotype in human mesenchymal cells incubated in presence of osteogenic factors. A stimulatory effect on osteoblastic factors secretion was observed after 3 hours of Bortezomib treatment. Consistently we found that Bortezomib significantly increased the activity of the transcription factor Runx2/Cbfa1 in human osteoblast progenitors without affecting the canonical WNT signaling pathway checked by the evaluation of nuclear and cytoplasmatic active beta-catenin levels. Consequently a stimulatory effect of Bortezomib on bone nodule formation could occur during Bortezomib treatment.

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**CO-069**

**NUCLEAR FACTOR-KAPPA B LOCALIZATION IN MULTIPLE MYELOMA PLASMACELLS AND MESSENCHIMAL CELLS**

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Nuclear factor-kappa B (NF-kB) is a multifunctional transcription factor that regulates different signal transduction pathways such as cell survival and proliferation. A number of tumors display activated (nuclear) NF-kB, which contributes to promote cancer cell growth and resistance to chemotherapeutic drugs. NF-kB has been shown to be constitutively active in multiple myeloma (MM) patients more than in normal individuals. A stimulatory effect on bone nodule formation process could occur during Bortezomib treatment.
tion for 24h. As for fresh MM cells, IDO expression in BMC was determined in 15 MM patients; our data show that IDO can be expressed in fresh BMC. 2 out of 3 IDO+ patients were examined at diagnosis. The presence of IDO protein is confirmed on the decalcified BM biopsies by immunohistochemistry with anti-IDO monoclonal antibody. 5/5 BMC were inducible by IFNg, 1/1 by PGE2. To distinguish the cell subset responsible for IDO expression, BMC isolated from 10 patients BMC have been tested for IDO mRNA transcription; 2/10 cases resulted IDO mRNA positive. Out of 9 CD157+ analysed, 2 resulted positive; IFNg induces IDO in all of them. The active specific immunotherapy requires all the conditions to generate antigen-specific immune responses (e.g., identification of tumor antigens, HLA restriction, and frequency of cytotoxic cells). On the contrary, relieve of negative immunoregulation is an easier approach simple to combine with chemotherapy. This research shows the IDO expression in MM BM microenvironment, potentially relevant to identify the Trp metabolism as a new target of biological interventions in MM.

CO-071
CLINICAL AND MOLECULAR RESULTS OF A POST-TRANSPLANT CONSOLIDATION WITH BORTEZOMIB, THALIDOMIDE AND DEXAMETHASONE IN MULTIPLE MYELOMA

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Introduction. Autologous stem cell transplant (ASCT) is unable to ensure disease eradication in Multiple Myeloma (MM). It is unknown whether the use of new agents following ASCT might ensure further cytoreduction allowing patients to enter molecular remission (MR) and whether this achievement might be of clinical relevance. We evaluated if a consolidation therapy with Bortezomib, Thalidomide and Dexamethasone (VTD) might impact the kinetic of minimal residual disease (MRD) and increase the rate of MR. Methods. Patients were eligible if they had a molecular marker and a documented complete remission (CR) or very good partial remission (VGPR) following ASCT. Each of the 4 cycles increase the rate of MR.

Methods.

CO-072
MAGNETIC RESONANCE IMAGING OF THE SPINE IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: PROGNOSTIC AND CLINICAL IMPLICATIONS

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Magnetic resonance imaging (MRI) has demonstrated to be more sensitive than plain x rays in detecting spinal involvement in multiple myeloma (MM) patients, as both vertebral lesions and pattern of bone marrow involvement (focal, diffuse or normal) can be depicted. In the present study we have prospectively evaluated the clinical and prognostic role of spinal MRI in 105 newly diagnosed MM patients (60M, 45E; median age = 56yrs) that had subsequently undergone cytoreduction and autologous stem cell transplant, either single (n=24) or double (n=81). Pattern of marrow involvement was focal in 64 cases (61%), diffuse in 25 (24%) and negative in 16 (15%). Patients with a diffuse pattern showed a significantly higher bone marrow plasma cell infiltration (p=0.05) and beta2 microglobulin values (p=0.04); 32% of them had a stage III disease according to ISS, in comparison to 9% and 0% of patients with a focal or negative pattern, respectively. Response rate to whole treatment program was similar in the three groups of patients, with a stringent defined CR obtained in 36% of patients with a focal pattern, 28% in those with a diffuse pattern and 31% in patients with a negative MRI. A focal pattern of bone marrow involvement was associated with a significantly higher probability of experiencing an overt vertebral lesion (73% vs 46% in patients with diffuse pattern, p=0.04), either a compression fracture or a vertebral mass. Consistent with this finding, also extraspinal bone lesions were more common in patients with a focal pattern (62%) as compared to patients with a diffuse or negative pattern (44% and 6% respectively). Furthermore, serum crosslaps were significantly increased in patients with a focal pattern (7027 pmol/L±655SE vs 5501 pmol/L±916SE in those with a diffuse pattern, p=0.04). According to our data, a diffuse pattern of bone marrow involvement could be predictive of a more aggressive disease, even though these data needs to be confirmed in a larger series. A focal pattern could help to identify MM patients more prone to advanced bone disease, so that a careful monitoring and bisphosphonate therapy should be recommended.
**INFECTIONS**

**CO-073**

**MOLECULAR EPIDEMIOLOGICAL INVESTIGATION OF AN OUTBREAK OF PSEUDOMONAS AERUGINOSA SEPSIS IN STEM CELL TRANSPLANTATION PATIENTS**


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Pseudomonas aeruginosa represents one of the main causes of nosocomial infections in immunocompromised patients, responsible for high morbidity and mortality. From May to September 2006, in the Bone Marrow Unit of the Careggi Hospital in Florence, Italy, 8 severe P. aeruginosa bloodstream infections were diagnosed in patients undergoing stem cell transplantation (3 autologous and 2 allogeneic transplants). All isolates resulted phenotypically similar (same biochemical pattern, same pattern of sensitivity to antibiotics). Three of the pts (2 pts with acute leukemia receiving allogeneic transplant and one pt with non Hodgkin Lymphoma receiving autologous transplantation) had shared the same room in different periods. In the hypothesis of a possible environmental source of the infection, samples for microbiological evaluation (phenotyping analysis, AFLP- amplified fragment-length polymorphism and arbitrarily primed PCR; AP-PCR) were collected from different sources that had potential for cross-contamination throughout the Bone Marrow transplantation Unit, including the electrolytic disinfectant used for medication of skin and mucosae (Irgasan) and facilities (5% Antisapril). A strain of Pseudomonas aeruginosa with the same phenotype of the one found in patients was isolated from the Irgasan soap. To confirm the hypothesis of the clonal origin of the isolates and of the transmission of the infection through the soap, a molecular typing by AFLP and AP-PCR was performed on the P. aeruginosa isolates from the 5 blood cultures and the soap. The AFLP analysis showed an internal similarity of 93% within the samples of the three pts sharing the same room and the soap. The results of AFLP analysis were compared with the AP-PCR analysis. The two methods provided similar conclusions about the clonal origin of these isolates. These data were highly suggestive of a single infection source and strongly suggested an environmental transmission route.

**CO-074**

**THE ENUMERATION OF ASPERGILUS SPECIFIC T-CELLS THROUGH AN EX VIVO ENZYME–LINKED IMMUNOSPOT ASSAY MAY BE AN EFFECTIVE TOOL IN THE DIAGNOSIS OF INVASIVE ASPERGILLOSIS IN HEMATOLOGIC PATIENTS**


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**Introduction.** Invasive Aspergillus (IA) has a poor outcome in hematologic patients (HP) because its prompt and undoubted diagnosis is hampered by unreliable diagnostic tools. Recent studies, either in mice or in humans, have suggested that Aspergillus-specific interferon-gamma-producing T-cells (IFN-gamma+T+1) are protective, while Aspergillus-specific interleukin-10-producing T-cells (IL-10+T+2) are permissive versus IA. We have evaluated whether enumeration of Aspergillus-specific IFN-gamma+T+1 and/or IL-10+T+2 through an ex vivo enzyme-linked immunospot (ELISPOT) assay may be an effective tool in the diagnosis of IA. **Methods.** ELISPOT was performed, as described [Potenza et al, Leukemia], on 20 HP and 5 healthy subjects (HS). Nineteen out of 20 HP presented pulmonary infiltrates (PI) at the high resolution computed tomography; whereas the remaining one showed multiple hepatic lesions (HL) at abdominal ultrasonography. A diagnosis of the PI and of liver lesions by histologic or cultural methods has been obtained for all the patients. In 12 out of 20 (60%) patients with PI, the galactomannan (GM) antigenemia has been also performed on bronchoalveolar lavage fluid (BAL). **Results.** The diagnosis of PI in the 19 patients were: bacterial pneumonia in 12, lymphomatous pneumonia in 1, granulomatous pneumonia in 2, and pulmonary IA in 4 cases. Liver IA was diagnosed in the patient with the HL. ELISPOT resulted positive in all 5 patients with proven IA either for Aspergillus-specific IFN-gamma+T+1 or IL-10+T+2; at each determination (sensitivity 100%). ELISPOT resulted negative in the remaining 15 patients with a pneumonia of non fungal etiology (specificity 100%). In the HS, ELISPOT resulted weakly positive only for IFN-gamma+T+1. GM resulted positive on BAL in 1 out of 4 patients with IA, and in 2 out of 8 patients with pneumonia of non fungal etiology (sensitivity 20%; specificity 75%). **Discussion.** The ELISPOT results in the HS were consistent with a previous study showing the production of IFN-gamma by of PBMCs from HS stimulated with Aspergillus antigens. Furthermore, in the 5 positive patients, ELISPOT has provided the description of the kinetics of the Aspergillus-specific human response in the course of an IA, in vivo, showing: a permissive immunity at the onset of disease and during progression and a protective one during stabilization/regression and after resolution. Our findings demonstrate the potential of ELISPOT in the diagnosis of IA and should induce further studies to validate this assay.

**CO-075**

**ENTEROCOCCI, VIRDANS STREPTOCOCCI AND PSEUDOMONAS SPP: TOWARDS A NEW BACTERIAL EPIDEMIOLOGICAL PATTERN IN HAEMATOLOGICAL UNITS. RESULTS OF A PROSPECTIVE SURVEILLANCE STUDY**


**Introduction.** Monitoring bacterial epidemiology is very useful for adopting an adequate antibiotic policy at haematological units. The aim of this study was to define the incidence of pathogens at our Institution and to identify emerging epidemiological patterns. **Methods.** During two consecutive 18-month periods (A: Apr2004-Sep2005; B: Oct2005-Mar2007), 1892 admissions to our Institution and all febrile episodes and/or infections were recorded. Infections were considered microbiologically proven (MPI) when microorganisms were isolated from infections sites. Patients were evaluated for underlying haematological disease, status of disease, neutropenia, antibiotic prophylaxis and presence of CVC. **Results.** MPI were 154 in period A and 161 in B. Bacteria represented 90% of all isolates, with a predominance of Gram-negative (G-) with respect to Gram-positive (G+) (A: 49% vs 41%; B: 46% vs 43%) both in period A and B. Among G-, Enterococci and virdans Streptococci increased during period B (respectively: 13/68, 19% vs 3/32, 9%; p=0.04) and 2/68, 3% vs 10/75, 13% (p=0.03); uncontrolled status of disease was a risk factor for Enterococci (p=0.046), whereas Streptococci occurred mainly in patients in complete or partial remission (p=0.04). Among G+, Pseudomonas spp increased in period B (17/81, 21% vs 39/79, 49%; p=0.01), particularly in patients out of prophylaxis (16/48, 33% vs 10/75, 14%, p=0.01). Antibiotic resistant strains increased in period B: (19/70 vs 3/32, p=0.007). Vancomycin resistant Enterococci (VRE) increased from 23% to 38%; 4 cases of fluoroquinolone resistant Streptococci and 5 of multiresistant Pseudomonas were observed in period B, as compared to none in period A. Death occurred in 4 (2 VRE) of 13 enterococcal infections in period A and in 2 (1 VRE) of 26 in B, being respectively 33% and 13% of all fatal MPI, in patients with uncontrolled status of disease. Two patients with streplococcal infections and 2 with Pseudomonas infections (1 multiresistant) died, both in period B (33% of all fatal MPI). 1 patient each showed controlled status of disease. **Discussion.** Epidemiological surveillance at our Institution showed the emergence of Enterococci, virdans Streptococci and Pseudomonas spp. Virdans Streptococci occurred more frequently in patients in complete or partial remission and may represent a life-threatening event. As antibiotic resistance is frequent and related crude mortality relevant, a careful policy for antibiotic use is warranted.
Epstein-Barr Virus (EBV) infection, a serious complication that can develop after either solid-organ or HSCT, is often associated with PTLD. EBV-LPD staging included physical examination and on serial quantitative and qualitative detection of EBV-DNA copies in blood by PCR. EBV-LPD staging included physical examination and on serial quantitative and qualitative detection of EBV-DNA copies in blood by PCR. EBV-LPD staging included physical examination and on serial quantitative and qualitative detection of EBV-DNA copies in blood by PCR.

Methods. The incidence of EBV-LPD was retrospectively assessed in recipients of allogeneic T cell-depleted hematopoietic stem cell transplantation. Between October 2006 and December 2006, 272 patients were have been transplanted from either HLA-matched (n=84) or HLA-mismatched donors (n=188). A diagnosis of EBV-LPD was based on tissue biopsy and/or whole-body CT scanning. EBV-LPD was confirmed at histology in 7 cases; in 2 patients (one with rhino-pharangeal mass and the other with meningel involvement) a biopsy specimen was difficult to obtain; the third patient had a rapid progression of the EBV-LPD and diagnosis was based on the clinical presentation and the fast increase in the peripheral blood EBV-DNA copies (range 843,437 copies EBV genome/mL). Risk factors for EBV-LPD were immunosuppression with cyclosporine and prednisone because of acute GvHD in 4/10; in the other 6 patients a low CD3+ cell count was the main risk factor. Therapy was mainly based on the combination of the anti-CD20 monoclonal antibody (Rituximab) with cidofovir in 9, DLI in 7 and CHOP-like chemotherapy in two. Four of the ten patients (40%) were non responders (3 died of EBV-LPD progression and one of relapse of the original disease). Six of the ten patients (60%) achieved a complete remission of the EBV-LPD (4 are still alive in CR, 2 died after the resolution of the EBV-LPD because of leukemia relapse and interstitial pneumonia, respectively). Conclusions. Avoiding any posttransplant immunosuppression after an extensively T-cell and B-cell-depleted matched or mismatched HSCT contributes to reduce the incidence of EBV-LPD. An early Rituximab-based treatment is effective in reducing mortality. In the presence of EBV-LPD, DLI is feasible without the risk of GvHD and helps to prevent late EBV reactivation.

Background. Post-transplant lymphoproliferative disorder (PTLD) is a serious complication that can develop after either solid-organ or hematopoietic stem cell transplantation (HSCT). A strong correlation between Epstein-Barr virus (EBV) infection, the grade and type of the immunosuppression and the development PTLD is well recognized. The incidence of PTLD after HSCT transplantation is generally lower respect to the solid organ one. However, the risk is significantly elevated in some groups of patients (unrelated and mismatched donors transplants, T-cell depleted) with important morbidity and mortality. The detection and quantification of EBV-DNA load in peripheral whole blood have been utilized as a prognostic marker for the development of PTLD. Aim of the study. To evaluated the EBV load as a parameter for the prediction and monitoring of PTLD. Methods. The diagnostic value of frequent monitoring EBV viral load from all HSCT patients visiting our hospital between January 2006 till now was investigated. EBV viral load was detected in peripheral whole blood by a quantitative real-time polymerase chain reaction (qRT-PCR) using the LightCycler Instrument (Roche Diagnostics, spa). In our study EBV DNA levels were determined in a range of 102 to 106 copies/mL Results. No patients were positive at the baseline PCR-EBV evaluation before HSCT. EBV reactivation was observed in 9 out of 17 (53%) HSCT patients: 5 (23%) and 12 (70%) patients had received the graft from an unrelated and a related donor respectively. None of our 17 HSCT patients developed PTLD during the study period. Anti-CD20 monoclonal antibody (rituximab) immunotherapy has been done in 2 patients without PTLD who received the graft from an unrelated donor when their EBV viral load exceeded the value of 5.000 EBV copies/mL. Conclusions. The pre-emptive modulation of immunosuppression after HSCT guided by EBV-DNA load appears to be a safe approach and a powerful diagnostic tool to monitor HSCT patients at risk of developing PTLD late after transplantation.

Fungal pneumonia is the most lethal infection in immunocompromised patients. We investigated the role of broncho-alveolar lavage (BAL) on diagnosis and outcome of 142 patients affected by haematological diseases, hospitalized in our clinic between January 2003 and July 2006, that underwent BAL examination. Methods. Of 142 patients, 20 (14%) had possible/probable fungal pneumonia. Of those 20, 15 underwent allogeneic T cell-depleted hematopoietic stem cell transplantation (allo-HSCT), 2 underwent autologous hematopoietic stem cell transplantation (auto-HSCT), 2 were treated by conventional chemotherapy and 1 with immunosuppressive therapy alone. BAL was performed because of a) slowly resolving pneumonia (n=10), b) broad spectrum anti-infective therapy resistant pneumonia (n=1), c) rapidly progressive pneumonia (n=6), d) relapsed pneumonia (n=1). 14/20 patient had relapse of the disease at the time of pneumonia. High Resolution thoracic CT (HR-CT) scan showed nodules, with or without halo sign, in 12 out of 20 patients; the remaining 8 patients showed aspecific infiltrates. Results. 11 patients were diagnosed with a probable fungal pneumonia (10 with Aspergillus spp found in the BAL liquid, 1 with Candida Parapsilosis found both in BAL and blood samples); the remaining 9 patients showed possible fungal infection (i.e., suggestive findings at HR-CT, but isolation of microbes with uncertain pathogenicity). Moreover, a virus was found at PCR analysis in 5/20 patients: 3 cases had Cytomegalovirus (CMV), 1 had parainfluenzavirus 1, 1 had both CMV and parainfluenzavirus 3/5 patients with both Aspergillus spp and virus died of pneumonia-related causes, suggesting an unfavorable combination. The overall pneumonia-related death rate was 5/20 patients with possible/probable fungal pneumonia. All 6 deaths occurred in the allo-HSCT cohort, and all had a fungal-viral coinfection (BAL and/or viremia). 5/6 deceased patients had a relapsing disease, and 2 were treated with immunosuppressive therapy because of severe GvHD. BAL results modified anti- infective treatment in 12/20 patients, despite changes 4/12 died. Where treatment was not influenced by BAL results (n=8), two patients died. Conclusions: BAL modified therapeutic strategy in more than a half of cases, decreasing the use of ineffective drugs, but didn’t impact positively on patients’ outcome, the latter depending more by co-existing factors (fungal-viral coinfection, disease status at the time of transplant).
**TREATMENT OF ACUTE INVASIVE ASPERGILLUS RHINOSINUSITIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES OR APLASTIC ANAEMIA: THE ROLE OF VORICONAZOLE**


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**Introduction.** Acute invasive Aspergillus rhinosinusitis (AIAR) is associated with a high mortality rate in patients with hematologic diseases. Voriconazole is a drug of choice in the treatment of invasive aspergillosis but it has been mainly investigated in patients with pulmonary localization and only in few cases of AIAR. **Methods.** We evaluated the outcomes of patients with haematological diseases diagnosed with AIAR after voriconazole was available at our center and used as first choice treatment in microbiologically documented Aspergillus infections (voriconazole period group, n =22) compared with a historical group of patients diagnosed with AIAR before voriconazole was available and treated with conventional or liposomal formulations of amphotericin B (AmB) (control group, n = 17). The time to clinical improvement, defined as significant reduction of pain and local inflammatory signs, from the start of antifungal therapy was also compared in the two groups. **Results.** At univariate analysis, patients of the voriconazole period group had improved, although not significant, 3-month survival rate compared to the control group (73% vs 47%; hazard ratio [HR] 0.46; 95% confidence interval [CI], 0.16-1.3; p=0.15). In multivariable models voriconazole period group (HR 0.16; 95% CI 0.04-0.57; p=0.005) and focal sinonasal localization of infection (HR 0.12, 95% CI 0.03-0.46; p=0.002) were significantly associated with reduced mortality independent of other prognostic variables. Time to clinical improvement was significantly shorter among patients of the voriconazole period group (HR, 3.97; 95% CI 1.41-11.16; p=0.009). **Discussion.** Our study seems to show a survival advantage of voriconazole compared to formulations of AmB in neutropenic patients with AIAR. The efficacy of this drug was also confirmed by the early clinical improvement of the signs and symptoms of sinonasal infection observed. High antimicrobial activity against all species of Aspergillus and pharmacokinetic properties could justify the advantages of voriconazole compared to AmB formulations in the treatment of AIAR.

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**MICROBIOLOGIC SURVEY WITH BIOTYPING METHODS IN HEAMATOLOGICAL PATIENTS**

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Infections are an important cause of morbidity and mortality in haematological patients. Molecular epidemiological survey is a very important tool to understand the nosocomial pattern of each hospital and to identify outbreaks. From 1 July 2006 to 31 March 2007 all patients admitted in our Hematology and Bone Marrow Transplant Unit were monitored to detect the appearance of suspected hospital-acquired infections and to detect the occurrence of multi-drug resistant bacteria (MDR). This study was performed by connecting the Laboratory Information System to the Vigi@ct System (Biomerieux) which is a software capable of providing real time alarms for suspected nosocomial infection and for presence of MDR. By Vigi@ct system was possible to obtain several epidemiology reports. A molecular typing by AFLP (amplified fragment-length polymorphism) on some of the more common pathogens (P. aeruginosa, E. coli, E. faecium, E. faecalis and S. aureus) was also performed in order to identify possible nosocomial outbreaks. We had detected 78 infections, 92 colonisations and 217 isolates. Among colonisations the most common organisms were C. albicans (29%), S. aureus (11%), K. pneumoniae (8%), P. aeruginosa (7%), E. coli (7%), S. epidermidis (6%), Enterobacter cloacae (4%), S. maltophilia (3%), S. marcescens 8%, E. faecalis (3%), C. glabrata (3%), others (16%). Among the infections, the most frequent sites were: blood (54 blood cultures), lung (8), central venous catheter (6) and other sites (13). Underlying malignancy of the patients with bloodstream infections (BSIs) were acute leukaemia (26), lymphoma (4), severe aplastic anaemia (1), myelodiplastic syndrome (1). We found BSIs in 6 allogenic and in 6 autologous transplants in the Transplant Unit. P. aeruginosa was isolated in 24% of BSIs, S. epidermidis in 20%, E. faecium in 18%, E. coli in 16%, Candida sp in 7%, others 15%. Among the 13 Paeruginosa BSIs, 5 were resistant to cef-tazidime (38%), 9 (69%) to quinolones, 4 (31%) to aminopenicillins, 1 (0.8%) to β-lactam- lactamase inhibitor, 1 (0.8%) to meropenem, but none to amikacine. Seventy percent of E. faecium strains were HLA R; 18% of the Enterobacteriaceae (2 E. Coli) were ESBLs producers and none of the staphylococci were slime producers. AFLP study showed a clonal origin of the isolates in three out of seven patients with P aeruginosa bloodstream infection undergoing BMT. Our results underline the importance of strict microbiological survey in high risk patients for early detection of nosocomial outbreaks employing biotyping methods.
EVI1 is a nuclear oncoprotein deregulated by recurring chromosomal abnormalities in MDS. The expression of this gene in MDS patients is considered a very poor prognostic marker and is associated with failure of normal erythropoiesis and with megakaryocytic defects. Erythropoiesis and megakaryopoiesis are controlled by the nuclear protein GATA1. We recently generated a mouse model of MDS by expressing EVI1 in murine BM and showed that the forced expression of this gene results in a fatal disease with features characteristic of MDS including dyserythropoiesis and fatal anemia. We also showed that EVI1 directly interacts with GATA1 and disrupts GATA1-bundling to DNA. Here we describe the effects of mutating the two zinc-finger motifs of EVI1, which form the interacting surface with GATA1. This EVI1 mutant, EVI1(1+6Mut), loses the ability to bind to and repress GATA1 in vitro and in cell lines, and GATA1 target genes that are repressed in presence of EVI1 are expressed at normal level in presence of EVI1(1+6Mut). To determine whether the block of normal erythropoiesis caused by EVI1 is alleviated by the mutations, we compared the differentiation response to Epo of normal BM progenitor cells and cells expressing either EVI1 or the EVI1(1+6Mut). The EVI1-expressing cells were virtually unable to generate erythroid colonies after Epo stimulation. In contrast, the EVI1(1+6Mut)-cells produced about 65% of the colonies formed by the control cells. All the EVI1-positive erythroid cells showed dysplastic features similar to those observed in MDS patients, including binucleate cells, chromatin bridges and nuclear-cytoplasmic maturative asynchronizations. These characteristics were less prominent in EVI1(1+6Mut)-positive cells and were observed only in a minority of cells. To determine whether disruption of EVI1-GATA1 interaction rescues erythropoiesis in vivo, we introduced either the wild type EVI1 or the EVI1(1+6Mut) mutant in mice by BM infection and transplantation. In contrast to the EVI1-positive mice, which succumb of pancytopenia and severe anemia 10-12 months after BM transplantation, the EVI1(1+6Mut)-positive mice are still alive 18 months after the BM transplantation and have normal PB counts. Analysis of one EVI1(1+6Mut)-positive mouse, which appeared to be healthy at the time of death 12 months after BM transplantation and was sacrificed for analysis, showed normal erythroid cells in the BM. As importantly, progenitor cells isolated from the BM of this animal had the ability to respond to and terminally differentiate in response to Epo or GM-CSF as control cells. Comparative analysis of peripheral blood smears revealed that the RBC defects observed in the EVI1-mice, such as anisokaryokaryosis and an increased number of polychromatophils, were very attenuated in EVI1(1+6Mut)-mice. Based on these results, we propose that when inappropriately expressed in the BM, EVI1 blocks erythroid differentiation by direct interaction with GATA1 leading to impairment of GATA1 gene regulation. We further propose that this differentiation block is a major cause of anemia in EVI1-positive MDS patients and results in loss of Epo response. Finally, we propose that the disruption of EVI1-GATA1 interaction could be a highly effective way to inhibit EVI1 in MDS. Taken together, our findings show that selective specific amino acid mutations rescue the normal functions of GATA1, allow Epo response (in a mouse system), and attenuate the erythroid dysplasia, and provide a novel and exciting new direction for designing therapeutic agents for EVI1-positive MDS patients.
CO-083
WT1 GENE EXPRESSION IS SIGNIFICANTLY INHIBITED BY VORINOSTAT AND BORTEZOMIB IN MYELOPROLIFERATIVE/MYELODYSPLASIS DISEASES
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Wilms’ tumor gene (WT1) has been reported to play a significant role in differentiation and leukemogenesis. In acute myeloid leukemia, high levels of WT1 have been associated with lower complete remission rates, reduced overall and disease-free survival. Moreover, this gene has been linked to myelodysplasia/myeloproliferative disorders. To note that vorinostat and bortezomib both inhibit NF-kB, whose activity has been regulated in the idiopathic myelofibrosis (IMF). On these bases, we evaluated WT1 mRNA levels in a myelodysplastic (P39) and in a megakaryoblastic (MO7-e) cell line, in order to test if treatment by a histone deacetylase inhibitor (vorinostat) and/or a proteasome inhibitor (borzetomib) could in vitro modulate the WT1 expression. Quantitative RT-PCR assays were performed on all samples to determine the expression of WT1. ABL was adopted as reference gene. In the MO7-e cell line, bortezomib, added at the IC50 (20 nM), reduced WT1 levels up to 16% (control=100%), when added to the culture at higher concentration (50 nM), WT1 levels decreased up to 7%. Vorinostat 1.5 µM (IC50) reduced WT1 expression to 28%, whereas as 5 µM WT1 down-regulation was higher (from 100% of the untreated cells up to 6%). Interestingly, when the two drugs were co-added to the culture, each one at its IC50, WT1 mRNA expression was decreased up to 5%, suggesting a synergistic action exerted by vorinostat and bortezomib. In P39 cell line, the anti-proliferative effect of bortezomib resulted higher than in the MO7-e (IC50=6 nM vs 20 nM); on the contrary, vorinostat IC50 was higher in the P39 cells (2.5 µM vs 1.5 µM). When bortezomib was added at the IC50, WT1 levels decreased up to 71%; when added at higher concentration (15 nM), WT1 levels decreased up to 22%. Vorinostat 2.5 µM (IC50) reduced WT1 expression to 40%, whereas 5 µM WT1 down-regulation was more pronounced (up to 65%). When co-administered, each one at 5 µM, WT1 mRNA expression was decreased up to 19%, so confirming the synergistic action of vorinostat and bortezomib already observed in MO7-e cell line. These results show a significant down-regulation of WT1 exerted by two new promising drugs in two different in vitro models of myelodysplastic/myeloproliferative disorders. To note that vorinostat and bortezomib both inhibit NF-kB, whose activity has been reported to be relevant in the pathogenesis both of MDS and of IMF, disorders that still lack a curative treatment.

CO-084
OVEREXPRESSION OF MATRIX METALLOPROTEINASES 2 AND 9 AND VASCULAR ENDOThelial GROWTH FACTOR IN BONE MARROW CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME: BIOLOGICAL AND CLINICAL RELEVANCE
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Angiogenic factors influence the growth and differentiation of hematopoietic cells in normal conditions and in hematologic malignancies. Most angiogenic factors appear to be secreted by hematopoietic cells, and they may have autocrine and paracrine regulatory effects on the hematopoietic system. Also matrix metalloproteinases (MMP), which are able to degrade all the protein components of the extracellular matrix, especially MMP-2 and MMP-9, play a role in angiogenesis. The expression of various angiogenesis mediators has been found to be altered in myelodysplastic syndrome (MDS) bone marrow and abnormal angiogenesis has been implicated in the pathogenesis of the disorder. We analyzed by immunocytochemistry MMP-2, MMP-9 and vascular endothelial growth factor (VEGF) expression in bone marrow cells from 153 patients with MDS (68 RA, 52 RARS, 51 RAEB, 5 RAEB-t, 17 CMML) and 52 non hematopoietic subjects, in order to evaluate whether abnormalities in their expression were associated with relevant laboratory or clinical findings. Moreover, a possible correlation was investigated between MMP or VEGF positivity and altered apoptosis or proliferator. In normal samples MMP-2 was detected in rare myeloid cells, NOH and VEGF in most maturing myeloid cells. In MDS MMP-2 and VEGF myeloid levels were higher than in controls (p<0.0001 and p=0.004 respectively); MMPs and VEGF were often coexpressed. Also many erythroblasts expressed MMP-2 and VEGF. There was a positive correlation between MMP-2 erythroblast expression and erythroid dysplasia (p=0.002) and an inverse correlation between MMP-2 or MMP-9 myeloid expression and blast cell percentage (p=0.05 and p=0.04 respectively). A positive correlation between VEGF levels and apoptotic rate, as evaluated by TUNEL technique, was observed. High MMP levels in myeloid cells were associated with longer overall survival (p=0.03) and evolution-free survival (p=0.04). In conclusion, we have demonstrated an abnormal MMP and VEGF expression profile in MDS bone marrow cells. The production and release of these proteins may influence hematopoietic cell behaviour, possibly by a paracrine induction of inflammatory pro-apoptotic cytokines from endothelial cells and macrophages. The detection of their deregulated expression in MDS may be important also from the clinical point of view: it may provide a useful tool for diagnosis, prognosis and a possible target for experimental treatments.
MOLECULAR CHARACTERIZATION OF PI-PLC-BETA1 IN MYELODYSPLASTIC SYNDROMES

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Introduction. Phospholipids are key regulators of a large number of cellular functions and do not act merely as structural components of biological membranes. Among the enzymes of the inositol lipid cycle, it is well known that PI-PLCbeta1 is a key enzyme in nuclear signal transduction, that is involved in many cellular processes, such as proliferation and differentiation. In particular, the involvement of the PI-PLCbeta1 in hematopoietic differentiation prompted us and others to investigate this signaling molecule in hematological malignancies, such as Myelodysplastic Syndromes (MDS). MDS are a group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis and a high risk of evolution into AML, although it is still unclear what are the molecular mechanisms of the evolution of MDS into AML. By using fluorescence in situ hybridization (FISH) analysis, we have previously evidenced (Lo Vasco et al., Leukemia, 2004) that, in MDS patients with normal GTC banding and a fatal outcome, the PI-PLCbeta1 gene undergoes a monoallelic and interstitial deletion. Methods. In the present study, we evaluated 45 patients affected by MDS, either at low risk or at high risk of evolution into AML. FISH analysis was performed on each MDS patient at the time of diagnosis, to determine the presence of PI-PLCbeta1, PI-PLCbeta4 and the 20p sub-telomeric region. Results. Interestingly, the deletion is present only in high-risk patients; low-risk MDS, usually having a much better outcome, do not show this genetic anomaly. In particular, 13/30 high risk MDS patients (43%), either with normal or altered karyotype, showed the mono-allelic deletion of the PI-PLCbeta1 gene, while both PI-PLCbeta4 and the 20p sub-telomeric region were normal in all the patients analyzed. Discussion. Taken together, our results suggest the possible involvement of PI-PLCbeta1 in the progression of MDS into AML and pave the way for a larger investigation, aimed at identifying a possible high-risk group among MDS patients.

THE ORAL IRON CHELATOR ICL670 IS A POTENT INHIBITOR OF NF-kappaB AND THIS ACTIVITY IS INDEPENDENT FROM IRON OVERLOAD IN MDS CELLS.

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Patients affected by Myelodysplastic Syndromes (MDS) undergo iron overload due to blood transfusions. Recently oral chelation is under evaluation to reduce iron induced organ damage. It was reported that iron chelation is able to reduce the HB and platelets transfusion requirement. Finally, it was reported that iron activates NF-kB through TNFα release. Recently it was demonstrated that NF-kBis abnormally activated not only in acute leukemias but also in MDS patients. The aim of the study was to evaluate the effects of the oral chelator ICL670 (Novartis) on NF-kB activity in order to identify a possible mechanism responsible for the observed reduced transfusion requirements during chelation therapy. After informed consent 20 BM samples were collected from MDS patients. Eight were RA, 8 RAEB, and 4 AML secondary to MDS (s-AML). 12 of the patients presented iron overload (evaluated by SQUID biomagnetic liver susceptometry) and high ferritin levels. The remaining 8 patients were collected at diagnosis before transfusions and they presented normal liver iron concentration (LIC) and ferritin levels. MNC cells were separated and incubated with 100 μM ICL670 for 5 hrs. Moreover, K562 and HL60 cells were analyzed as control. Incubated control cells were evaluated for NF-kB activity using both EMSA and ELISA method. We detected an increased activation of NF-kB as compared to healthy subjects in 4 out of 8 RA 6 out of 8 RAEB, in all the cases of s-AML and in cell lines. No significant difference was detected in healthy subjects between RA and RAEB. After incubation with ICL670 a significant reduction of NF-kB was observed (p=0.0002). No significant difference was detected in NF-kB inhibition comparing patients with or without iron overload. In addition, ICL670 also inhibits NF-kB activity in HL60 and K562 cells. We concluded NF-kB is abnormally activated in MDS patients and this is not apparently related to iron overload being present in many patients before transfusion with normal ferritin levels and in cell lines. ICL670 acts as a potent NF-kB inhibitor and this property could explain the activity on BM cells which results in the improvement of the HB and platelets levels. This latter effect seems to be independent from the reduction of iron storage induced by oral chelation.

AZACITIDINE IN COMBINATION WITH EPO+G-CSF AND VALPROIC ACID RAPIDLY DETERMINES HEMATOLOGICAL IMPROVEMENT IN PRETREATED NON RESPONSIVE IPSS INT-1 MDS PATIENTS

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The DNMTinhibitor azacitidine has been approved by FDA for treatment of patients with myelodysplastic syndromes (MDS) of all IPSS risk scores. In Europe approval will be subject to results obtained in the ongoing phase III study involving only INT-2 and high risk MDS patients. In fact, a large number of MDS patients with lower IPSS score could be advantageously treated with azacitidine alone or combined with other agents, but data focused on this specific subset of patients are lacking. We evaluated whether azacitidine, in combination with growth factors and the histone deacetylase (HDAC) inhibitor valproic acid (VPA) could determine haematological response in pretreated, refractory MDS patients with IPSS score INT-1. We treated 9 patients with azacitidine 50 mg/m²/day for five days, plus erythropoietin (EPO) 40,000 U twice weekly, granulocyte colony stimulating factor (G-CSF) 300 mg, once weekly and VPA 600-1200 mg/day. These patients were not eligible for treatment with DNMTinhibitors in any of the ongoing trials and all of them had undergone previous treatments: EPO plus G-CSF for more than 24 weeks, without any hematological response. One of six had been treated also with thalidomide 100 mg/day, but no response was observed, due to intolerance to treatment and subsequent early drop out. All patients were RBC transfusion dependent, 4/9 both for RBC and platelets. Mean age was 62.2 (54-80). None of the patients had more than 6% bone marrow blasts and only 1/6 had a complex karyotype. Because of the heavy burden of transfusions and deteriorating general conditions, we started treatment. Courses were very well tolerated, with only nausea grade 1-2; in particular, the slow escalation in VPA doses prevented CNS side effect. VPA blood concentrations were kept within neurological therapeutic range. At present, patients received 4-10 courses of therapy, and all showed haematological improvement (1/9 CR). In particular, RBC and platelet transfusion independence or significant reduction in transfusion requirement was achieved for 9/9 patients, with also general reversal of neutropenia. In one patient, starting with platelets counts below 10x10⁹/L and regularly transfused weekly, platelet number was within normal range after 2 cycles of therapy. Azacitidine was used at lower doses than in CALGB and Phase III trials, according to recent evidence of efficacy of the drug, even at 50 mg/m²/die. Combination treatment with low dose-azacitidine, growth factors and the HDACI VPA was safe and well tolerated, and was extremely effective in inducing rapid haematological improvement in the entire (small) cohort of pretreated, resistant INT-1 risk MDS patients analyzed.
LABORATORY TECHNIQUES

CO-089
A PDGFRB-POSITIVE ACUTE MYELOID MALIGNANCY WITH A T(5;12)(Q33:P13.3) INVOLVING THE ERC1 GENE

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Background. In myelodysplastic syndromes/chronic myeloproliferative disorders a t(5;12)(q33;p13) PDGFRB correlates with male predominance, splenomegaly, peripheral blood and/or bone marrow eosinophilia, and sensitivity to imatinib mesylate. We report on the molecular characterization of a new t(5;12)(q33;p13) translocation.

Methods and Patient. An acute myeloid leukemia (AML) was diagnosed in a 36 year old man in October 2002. In November 2006 the AML relapsed 4 years after chemotherapy and autologous bone marrow stem cell transplant. Methods. Conventional cytogenetics was performed using standard procedures. Fluorescence in situ hybridization (FISH) was done with cosmids 9-4 and 4-1 for PDGFRB and with cosmids 179A6 and 148B6 for ETV6. DNA clones for loci/genes telomeric to PDGFRB were from clone RP11-1087E18.

Results. The karyotype was 46.XY,t(5;12)(q33;p13.3)[9]/46.XY,t(5;12)(q33;p13.3)[12]. FISH indicated that the PDGFRB gene was disrupted. The 12p13.3 breakpoint mapped telomeric to ETV6 were also used. The patient’s RNA was extracted with Trizol and retro-transcribed using the thermoscript RT-PCR System (Invitrogen). The first amplification round was performed with primers ERC1_1756 ex7F and CTGAGAGCTCCTGACTG-3’ and PDGFRB_2298R 5’-TAGATGGTCTCCCTTTGGTG-3’ and the second with primers ERC1_2288 ex11F 5’-CTCTTCTCTCGGATCTCGAAG-3’ and PDGFRBR1 5’-TAAAGCTTCTAGCCACT-3’. Results. The karyotype was 46.XY,t(5;12)(q33;p13.3)[9]/46.XY,t(5;12)(q33;p13.3)[12]. FISH indicated that the PDGFRB gene was disrupted. The 12p13.3 breakpoint mapped telomeric to ETV6. Probes for 12p13 narrowed the breakpoint to within clone RP1-1087E18 which encompasses ERC1 (derived from P3H13). Nested reverse (RT)-PCR amplified an ERC1/PDGFRB fusion transcript. Sequence analysis showed that nucleotide 3021 (ERC1 exon 15) was involved in an in-frame fusion with nucleotide 1837(PDGFRB exon 10) and in an out-of-frame fusion with nucleotide 2049 (PDGFRB exon 11). RT-PCR detected a minimal residual disease despite of haematological and FISH remission following chemotherapy.

Conclusions. The fusion transcript is progressively decreasing under treatment with imatinib mesylate. The observation of alternative RNA splicing restricted to the coding exons only may overlook such pathogenic mutations, this finding has implications in designing the strategy of analysis of the families with suspected FHL. Identification of Munc13-4 mutations allows genetic diagnosis of FHL3 in children and young adults, with immediate therapeutic implication including indication to HSCT.

CO-091
INTEGRATION OF THALIDOMIDE INTO UP-FRONT DOUBLE AUTOGLOUS TRANSPLANTATION FOR MULTIPLE MYELOMA: PROGNOSTIC RELEVANCE OF BASELINE CYTOGENETIC ABNORMALITIES

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Objectives. Thalidomide has been shown to be active in refractory multiple myeloma (MM). In the 1990s, two trials showed that the addition of thalidomide to standard treatment increased both response rate and overall survival. In a subsequent study, thalidomide was added to high-dose therapy as a consolidation in multiple myeloma patients with adverse cytogenetic abnormalities who relapsed after autologous transplantation. Analyses of high-dose MDTX and HSCT regimens showed that patients with karyotype abnormalities have a decreased survival in terms of increased response rate (p=0.01) and EFS (p=0.02).

Methods. We have evaluated 51 patients with advanced MM who were randomized to receive a double autologous transplantation (ASCT) with or without thalidomide as front-line therapy for patients with multiple myeloma (MM). Thalidomide (200 mg/m²) and dexamethasone (40 mg/d × 4d every month) were administered from the output until the day before the second ASCT. An analysis was performed on 311 patients who entered the thalidomide arm and were followed for a median of 22 months. Results. The rate of at least near complete response (nCR) increased from 19% after induction therapy up to 49% after the second ASCT. Transplantation-related mortality after the first and second ASCT was 1% and 3%, respectively. Median durations of relapse-free survival (RFS) and event-free survival (EFS) were 52 and 42 months, respectively. The 5-year projected overall survival (OS) rate was 70%. A case-control comparison of 135 of these patients with an equal number of pair mates who were assigned to receive double ASCT without incorporation of thalidomide showed that the significant benefits offered by thalidomide were reflected in patients with karyotype abnormalities.

Conclusions. Our results confirm the benefit of thalidomide in MM patients with adverse cytogenetic abnormalities. Thalidomide has proven to be effective in improving response rate and overall survival in patients with adverse cytogenetic abnormalities who relapsed after autologous transplantation.

CO-090
SPICING ERRORS OF MUNC13-4 ARE A COMMON CAUSE OF FAMILIAL HEMOPHAGOCYTOSIS SYNDROMES TYPE 3


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Introduction. Familial hemophagocytic lymphohistiocytosis (FHL) is a genetically heterogeneous disorder characterized by constitutive defects in cellular cytotoxicity resulting in fever, hepatosplenomegaly, cytopenia, and the outcome is fatal unless treated by chemo-immunotherapy followed by hematopoietic stem cell transplantation (HSCT). Mutations in the Munc13-4 gene giving rise to this disease have been identified, however these only account for 40% of cases. Lack of a genetic marker hampers the diagnosis, suitability for transplantation, selection of familial donors, identification of carriers, genetic counselling and prenatal diagnosis. Mutations in the Munc13-4 gene have recently been described in patients defined as with FHL3. The role of mutation causing alternative splicing sites is currently a focus of investigations in different human diseases. Methods. We sequenced the 32 exons and the adjacent intronic regions of the Munc13-4 gene in all patients with FHLH not due to PRFI mutations. Results. We identified 39 families with FHL3 due to biallelic Munc13-4 mutations. Of them, 20 had at least one mutation responsible for a splicing error. Two mutations, 753+1G>T and 1389+1G>A, had been previously reported; six mutations were instead originally contributed by our group: the 610A>G (M204V) and 1847A>G (E616G) which were recently described (Santoro et al. JMG 2006), and the previously unreported 117+59C>T, 952-1G>A, 1992+5C>A, 2449-12insC. To better understand the role of nucleotide substitutions predicted to affect splicing, we sequenced CDNA from some selected cases. The 1847A>G substitution, located two nucleotides from the end of exon 20, caused an abnormal splicing of exon 20 with a deletions of nine amino acids; the 753+1G>T, a splice recognition site, caused the deletion of the whole exon 9; the more profound intron substitution 117+59C>T caused an abnormal splicing of exon 2, probably introducing a additional criptic splice site. Conclusions. At difference with what observed in FHL2 and FHL4, splicing errors are a very frequent cause of disruption of the Munc13-4 protein, leading to FHL3. Unexpectedly, profound intron mutations, between +5 and +59, were documented as responsible of alternative RNA splicing. Analysis restricted to the coding exons only may overlook such pathogenic mutations; this finding has implications in designing the strategy of analysis of the families with suspected FHL. Identification of Munc13-4 mutations allows genetic diagnosis of FHL3 in children and young adults, with immediate therapeutic implication including indication to HSCT.
patients who lacked both del13 and t(4;14) had significantly longer EFS and OS than patients who carried either a single genetic abnormality or both of them (EFS: 54% at 5 years vs median values of 29 and 21 months, respectively; \( p < .001 \)); (OS: 81.5% vs 66% at 5 years vs a median of 41 months, respectively; \( p = .003 \)). In a multivariate analysis performed on an ITT basis, patients who lacked both abnormalities had similar probabilities to attain at least nCR than patients who carried either del13 or t(4;14) (50% vs 41%, respectively; \( p = .09 \)). Conversely, the presence of both these abnormalities adversely affected the rate of at least nCR in comparison with the absence of both genetic alterations (\( p = .001 \)). The most important and independent variable significantly extending EFS and OS was attainment of at least nCR (\( p < .001 \) for both); conversely, the presence of del13 adversely affected EFS (\( p = .006 \)). In conclusion, the addition of thal to double ASCT improved the response rate, RFS and EFS among MM patients. EFS was significantly shorter in the presence of del13, whereas both EFS and OS were positively affected by nCR.

**CO-092 GENOTYPE-PHENOTYPE STUdy of Familial hemophagocytic lymphohistiocytosis due to perforin mutations**

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Introduction. SM is characterized by activating mutations of Kit tyrosine kinase. While the D816V mutation renders Kit resistant to imatinib, regulatory type mutations are sensitive to inhibition. Kit mutations screening with sensitive methods is important for an appropriate therapeutic management of SM. Our aims were: to set up a D-HPLC-based screening method for mutations in critical regions of Kit; to assess the sensitivity and reliability of our D-HPLC assay as compared to RFLP analysis; to characterize additional mutations. All patients were screened in parallel by D-HPLC assay and by RFLP assay. In case of a positive D-HPLC signal, direct sequencing was performed to confirm the presence of a mutation. The PCR product was digested with the restriction enzyme HinfI to detect the nucleotide change at codon 516, leading to D816V mutation. For each sample scored as wild-type by D-HPLC and by RFLP analysis, a PCR product of 550 bp, corresponding to the transmembrane domain (TM) and to the juxtamembrane domain (JM), was screened by D-HPLC combined with direct sequencing. Results. By RFLP analysis 54/51 pts were positive for the D816V. By D-HPLC analysis, an abnormal elution profile was seen in 36/51 pts – all the 34 RFLP-positive cases as well as two additional pts. Direct sequencing confirmed the presence of the D816V mutation in 32 pts and, by RFLP assay, in 14 other pts. No additional mutations were found in 15 pts. In 20 pts scored positive by D-HPLC but negative by RFLP were found to have the J796I polymorphism. The 15 pts who did not harbour mutations in the J796I were further investigated by D-HPLC analysis of a RT-PCR product spanning the TM and JM domains. D-HPLC showed an abnormal elution profile in 5 pts. By direct sequencing one patient with the K546K mutation in the TM domain and one patient with the L460F mutation in the JM domain were reported to have a Kit mutation in the TM domain. Discussion. Our D-HPLC-based assay proved a straightforward, reliable and sensitive method for Kit mutation analysis. Furthermore, our D-HPLC-based screening method highlighted the importance of screening for mutations other than the D816V, mainly because the function of Kit regions, such as the TM domain, is still unclear. Supported by: European LeukemiaNet, COFIN 2003, AIL, AIRC, Fondazione del Monte di Bologna e Ravenna.

**CO-094 QUANTITATIVE MOLECULAR EXPRESSION OF THE IMMUNOREGULATORY ENZYME INDOLEAMINE 2,3-DIOXYGENASE IN ACUTE MYELOID LEUKEMIA CELLS AS A POSSIBLE MARKER FOR MINIMAL RESIDUAL DISEASE DETECTION.**

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The expression of the catalytic enzyme of tryptophan, indoleamine 2,3 dioxygenase (IDO) has been identified as a T-cell inhibitor effector pathway in different normal and neoplastic cells. We have recently shown that normal bone marrow (BM) cells, including hematopoietic CD34+ cells, express IDO mRNA only upon IFN-γ stimulation, whereas in a subset of human acute myeloid leukemia cells (AML) IDO is constitutively expressed both at molecular and protein level and induces immunological escape by promoting the generation of T-reg cells (Curti et al., Leukemia 2007; Curti et al., Blood 2007). To investigate whether
the IDO transcript can be used as a marker for minimal residual disease (MRD) detection, we use a real-time reverse transcription-polymerase reaction assay (qRT-PCR) to quantify IDO mRNA levels in peripheral blood (PB) and BM samples of newly diagnosed AML patients. The level of IDO transcript was evaluated as IDO copy number/10^4 ABL. As control samples, we used normal PB and BM mononuclear cells (MNCs). Our data showed that normal BM cells, including CD34+ cells, scored negative (i.e. the transcript was not detectable either by qualitative and quantitative RT-PCR), while normal PB controls expressed minimal amount of IDO mRNA (range 126-890). Among AML samples, we identified three subsets of patients according to IDO mRNA expression: 1) 22/46 (47.8%) IDO negative (i.e. range < 100), 14/46 (30.4%) IDO low (range 100-904) and 10/46 (21.7%) IDO high (range 1328-566051). BM and PB AML blasts gave similar results. Assessment of protein expression and enzymatic activity was in accordance with molecular results. Some patients were evaluated for IDO mRNA expression before and after induction chemotherapy and the IDO levels were found to correlate with the reduction of BM blasts. Taken together, our qRT-PCR data demonstrate that 1) normal PB and BM cells are negative for IDO mRNA expression, which, in turn, is significantly up-regulated in a subset of AML patients (IDO high) and 2) IDO mRNA expression correlates with tumor burden, thus suggesting its possible role in the detection of MRD in IDO high patients.

**CO-095**

**CLINICAL AND BIOLOGICAL PREDICTIVITY OF LH750 LYMPHOCYTE POSITIONAL PARAMETERS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)**


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**Introduction.** Several studies have been carried out to identify easily assessable laboratory parameters from automated analysers to obtain prognostic informations from circulating lymphocytes in CLL patients (French Coop Group CLL, 1990; Montserrat, 1988, Lanza, 1992). The Coulter cytometer LH750 performs WBC analysis using VCS technology (volume, light laser scatter and conductivity). As further information the analyser provides the leukocyte positional parameters (PP) as mean and standard deviation values of V (dcmn and dcsd) C (opmn and opsd) and light laser scatter (rlsmn and rlssd). MedCalc program was used for statistical analysis. **Results.** MN and CLL show significantly increased lyopmn (111) and lydcsd (25 and 21 respectively), and reduced lyrlsmn (69 and 72 respectively) (normal reference population: lyopmn 107, lydcsd 14, lyrlsmn 79); MN has lyrlssd (22) significantly increased respect to both normal subjects (18) and CLL (19). Using ROC curves analysis, 16 for lydcsd and 21 for lyrlssd result the best cut-off values to distinguish between normal and pathological samples and reactive (MN) and clonal (CLL) lymphocytosis, respectively (Figure 1A-B). Patients in Binet stage A show lower levels of lydcsd (20) than patients in other stages (25) (p=0.003). Trisomy 12 is associated with the highest lydcmn (103), in comparison with other cytogenetic aberrations (range 77-82) (Figure 2A). Patients carrying -17p and unmutated IgVH have the lowest lyrlsmn (63) than others (range 70-82) (Figure 2B).

**Methods.** We examined lymphocyte PP (LPP) in 87 CLL patients, 67 M, 20 F, median age 68,5 (69-88), 50 healthy subjects, 27 M, 23 F, median age 46 (18-65) as reference normal population, and 10 patients affected by infectious mononucleosis (MN), 7 M, 3 F, median age 18,5 (15-28). Peripheral blood samples were analysed in LH750 within 6 hours from withdrawal. CLL patients were classified according to Binet clinical stage, genomic aberrations (FISH for 13q-, 17p-, 11q- and trisomy 12), IgVH mutational status (PCR and sequencing) and ZAP-70 and CD38 expression (flow-cytometry). Unmutated IgVH genes status has higher lyopmn (114) than mutated one (110) (p=0.001).

**Conclusions.** In our study we confirm (Silva2006) the utility of LPP for MRD (≤16) and pathological samples with a sensitivity of 86% and a specificity of 98%; the value of 21 for lyrlssd discriminates between MN (≤21) and CLL with a sensitivity of 79% and a specificity of 80%.
NEOPLASTIC CIRCULATING ENDOTHELIAL CELLS IN HEMATOLOGIC MALIGNANCIES


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Abstract. Several studies have shown that bone marrow-derived endothelial cells (EC) may contribute to tumor angiogenesis and that in the peripheral blood of cancer patients there is an increased amount of circulating ECs (CECs) that may participate to new vessel formation. Recent data also showed that microvascular ECs in B-cell lymphomas are in part tumor-related reflecting a novel aspect of tumor angiogenesis. All together these observations suggest that tumors can elicit the sprouting of new vessels from existing capillaries through the secretion of angiogenic factors and that, in some cases, cancer cells can also mimic the activities of ECs by participating in the formation of vascular-like networks. To clarify if, in different hematologic malignancies with known cytogenetic aberrations, CECs are tumor-derived. We studied 21 patients with different hematologic malignancies (6 MM, 2 CML, 5 AML, 1 ALL and 7 CLL). To isolated CECs, we used a dual step immunomagnetic sorting by means of CD45 and CD144 antibodies. By using immuno-magnetic sorting in combination with CD45, we first eliminated all hematopoietic cells, which are CD45 positive, without affecting the EC component, which is characteristically CD45 negative. We then sorted CECs by means of CD146, an antigen expressed almost exclusively on ECs and absent on hematopoietic cells. To confirm the EC commitment, we then performed additional phenotypic studies with antibodies recognizing endothelial and neoplastic cells. FISH analysis was finally performed on sorted CECs with different commercially available probes in dual colour experiments. In all experiments more than 95% of immunomagnetically sorted cells were of EC origin as demonstrated by phenotypic analyses. After immunomagnetic selection less than 0.5% of cells were CD45 positive, while CD14 was expressed in 0.1% of all immunomagnetic sorted cells. More than 95% of immunomagnetically sorted CECs expressed VEGFR2, vWF, CD144 and UEA-1 lectin. Very few immuno-magnetically sorted CECs expressed antigens expressed on neoplastic cells (CD138, CD38, CD33, CD19, CD5). FISH analysis showed that a significant proportion of CECs was tumor-derived because they harbored the same genetic lesion as observed in neoplastic cells. The fraction of CECs showing the cytogenetic aberration averaged 20% (range, 11-34%); 200 cells observed in each case). The majority (>55%) of CECs presented features of EPCs because they expressed CD133, a marker gradually lost during EC differentiation and absent in mature ECs. Overall, 98% of CECs with genetic lesions were CD133 positive. Unsupervised gene expression profiling analysis suggests that similarities exist with normal endothelial progenitor cells in indolent forms of CLL. These findings suggest that in hematologic malignancies CECs are in part tumor related and with EPC features. These CECs may contribute to tumor neovasculogenesis and possibly to the spreading and progression of the disease. It is possible to speculate that neoplastic CECs may have arisen from a common hemangioblast precursor that can give rise to both neoplastic cells and ECs or alternatively through a process of dedifferentiation of a already committed cell into a cell with EPC characteristics followed by a redifferentiation into a terminally differentiated EC. Disguised neoplastic cells may then mimic functional CECs and contribute to tumor neovasculogenesis.

LONG-TERM MOLECULAR RESPONSES TO IMATINIB IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA: COMPARISON BETWEEN PATIENTS TREATED IN EARLY AND IN LATE CHRONIC PHASE


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Introduction. Achieving a major molecular response (MMoR) is the goal of chronic myeloid leukemia (CML) therapy. Indeed, patients (pts) who obtain a complete cytogenetic response (CCgR) may harbor different degrees of molecular disease, which are associated with different probabilities of event-free survival and durability of the CCgR. For a better analysis of the response to imatinib (IM) in pts treated in late chronic phase (LCP) we compared the pattern and the magnitude of molecular response of 115 LCP CML pts and of 60 early CP (ECP) pts in continuous CCgR.

Methods. Patients were monitored for conventional cytogenetic and molecular response every 6 months (mos). Molecular response was assessed on peripheral blood samples by RQ-PCR (TaqMan). Major molecular response (MMoR) was defined as BCR-ABL/ABL% below 0.05, whereas undetectable BCR-ABL transcript was defined as BCR-ABL/ABL% below 0.001%. Results. 295 pts were treated with IM after failure of interferon-alpha; 124 (42%) are still in stable CCgR and 115/124 are evaluable for molecular response at 60 mos. 76 ECP CML patients were treated with imatinib and a variable pegylated IFN-alpha dose (50, 100 and 150 microg/wk), which has been discontinued by 75/76 pts after a median time of 10 mos (range: 1-49); 63 (85%) pts are in continuous CCgR and 60/63 are evaluable for molecular response at 60 mos. In ECP pts, the frequency of MMoR was already very high at 6 months (63%, vs 88% in LCP pts) with a total rate of MMoR and undetectable levels of the BCR-ABL transcript of 78% (vs 48%), which increased up to 94% and 98% at 12 and 24 months, and remained substantially stable at subsequent evaluations. The proportion of LCP pts with this magnitude of molecular response was lower but increased over time (56%, 79%, 92% at 12, 24 and 48-60 mos). During follow-up, MMoR was stable in 88% and in 78% of early and late CP patients, respectively.Conclusions. IM induced a very high rate of MMoR either in early and in late CP pts. Although both ECP and LCP pts were in stable CCgR, RQ-PCR analysis showed that ECP pts obtained earlier, higher and more durable molecular responses, even in the long term. Acknowledgments. COFIN 2005, HRB 2001, AIRC, CNR, Fondazione del Monte di Bologna e Ravenna, European LeukemiaNet, AIL.
CO-099
THE MTOR INHIBITOR EVEROLIMUS COMPLEMENTS PRO-APOTOTIC AND ANTI-
Proliferative EFFECTS OF IMatinib IN CHRONIC MYELOID Leukemia
PROGENITORS BY PROMOTING THE NUCLEAR IMPORT OF NORMAL C-ABL PROTEIN
Mancini M,1 Zuffa E,1 Corrado P,1 Pagnotta E,1 Brusa G,2 Corradi V,1
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Deregulated proliferation and extended life expectancy of Chronic Myeloid Leukemia (CML) progenitors are driven by the constitutive tyrosine kinase (TK) activity of p210 Bcr-Abl protein. Still, the basis of residual, normal c-Ab1 loss of function associated with Bcr-Abl rearrangement remain elusive. Our work moved from the observation that p210 TK precludes the nuclear accumulation of c-Abl protein in response to genotoxic damage by preventing the disruption of its ligand to 14-3-3 scaffolding proteins. In Bcr-Abl-transduced 52D cell clones and CD34+ hematopoietic progenitors from CML patients at clinical diagnosis p210 TK inhibition in response to 24 hr in vitro exposure to 1 µM Imatinib (IM) was followed by 14-3-3-sigma phosphorylation, c-Abl release and nuclear import, apoptotic death and growth arrest. The last three events were further enhanced by complementary inhibition of 14-3-3 binding site by R18 peptide, further supporting the p210 TK negative impact on normal c-Abl function arising, at least in part, from the effects of fusion protein enzymatic activity on 14-3-3-sigma. MTor, a component of PI3k/AKT axis involved in 5 Cap-dependent initiation of translation, is a downstream target of c-Abl. The balance between mTor inhibition (proceeding from c-Abl protein repartitioning) and activation (promoted by p58 MAP kinase phosphorylation of Tuberous Sclerosis 2 gene protein, Tuberin) and enhanced binding to 14-3-3) in response to Imatinib, may be critical for the outcome of drug resistance. Accordingly, mTor inhibition in response to in vitro exposure to Everolimus (100 nanoM) complements IM pro-apototic and anti-proliferative effects on CML progenitors through phosphorylation of c-Jun-N-terminal kinase (JNK) and dephosphorylation of p38 Map kinase and Tuberin. In conclusion, our results support the advantage of combined P210 TK and mTor inhibitors in circumventing the activation of a compensatory pathway for the persistence of Bcr-Abl-rearranged cells in IM-treated CML patients.

CO-100
MULTIPLE MOLECULAR MECHANISM MAY ACCOUNT FOR RESISTANCE TO IMatinib IN RESISTANT CELL LINES
Esposito N,1 Izzo B,2 Quantaroli F,1 Colavita I,2 Buonomo T,1
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Ruoppolo M,1 Rotoli B,1 Pane F2
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Imatinib mesylate, given its high activity against chronic myeloid leukemia (CML), is the current first-line therapy for the treatment of chronic phase (CP) CML. Moreover, some patients are rendered resistant to the treatment even after complete cytogenetic remission (CCgR), however, the mechanisms underlying this resistance remain elusive. Our work moved from the observation that p210 TK inhibition in response to 24 hr exposure 187 living pts was 72 mos (range, 48-79). Median age was 52 yrs (range: 19-82). Median duration of CP prior to IM was 38 mos (range: 1-202). 158 pts (53%) achieved a CCgR, 106 (69%, early responders) within 12 mos and 47 pts (31%, late responders) thereafter. 34 pts (22%) lost the CCgR, 25 (67%) within 24 mos from the date of its first achievement. The CCgR loss rate was 20% and 25% in early and late responders (p=0.25). 115/124 pts with stable CCgR were evaluable for molecular response. The frequency of M0MoIR increased during follow-up (38%, 46%, 53% at 6, 12 and 24 mos) up to 70% at 36 and 60 mos. M0MoIR was either unstable (17% of cases) or stable (38%), and was occasionally undetectable (10% of cases). However, a partial response in chronic phase was observed in 8 (5%) cases, with 2 pts becoming and remaining molecularly negative. The proportion of pts with undetectable RT-PCR remained low throughout (7 to 16%). 13 pts underwent an allogeneic transplant, 12 are alive in AP and 72 (26%) have died (36 in BC). OS and PFS at 5 years were 77% and 72% for all pts and 90% and 89% for the pts who achieved a CCgR, with no differences between early and late responders. Conclusions. This is the first report on the 6-year outcome of LCP pts, whose number is substantial in the countries where IM became available years ago, and is even greater in many other countries where IM became available later on, or where IM is not yet available. For pts who achieved a CCgR, PFS and OS at 6 years likely to be as good as for ECP pts; moreover, IM determined a high and early frequency of M0MoIR, which increased over time. Acknowledgments. COFIN 2003, FIRB 2001, AIRC, CNR, Fondazione del Monte di Bologna and Ravenna, European LeukemiaNet, AIL.
A PROSPECTIVE STUDY IN PH+ CML PATIENTS: FISH IS EFFECTIVE AS CONVENTIONAL CYTOGENETICS FOR DEFINITION OF CYTOGENETIC RESPONSE TO IMATINIB. CORRELATION WITH MOLECULAR RESPONSE (A GIEMSA WP ANALYSIS)

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Introduction. We planned a prospective analysis involving 3 multicentric national studies of the GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Adulto) CML Working Party (WP) to evaluate the correlation between conventional cytogenetics (CC) and FISH response in Philadelphia positive (Ph+) chronic myeloid leukemia (CML) patients in chronic phase (CP) treated with imatinib. Methods. Karyotype and FISH analyses were performed on bone marrow cells in 26 local laboratories and in 12 WP reference labs. Cytogenetic examinations were performed at enrollment, after 3 (in 1 study), 6 and 12 months of treatment. Peripheral blood samples for quantitative RT-PCR were centralized in Bologna at enrollment, after 3, 6 and 12 months on imatinib. Results. A strong correlation between CC and FISH was observed (r=0.91, p=0.008). Table shows the demonstration of FISH data according to CC data and the number of metaphases available for CC. Of 263 patients (pts) in CCgR by CC and > 20 metaphases observed, 79.5% were FISH negative, 16.7% showed a low rate of FISH positive cells (1-5%) and 3.8% an higher rate. Of 102 pts in CCgR by CC but with < 20 metaphases observed, 72.5% were FISH negative, 20.7% showed a low rate of FISH positive cells (1-5%) and 6.8% an higher rate. Of 80 pts in PCgR by CC, 52% were FISH positive with a superior amount of positive cells. Moreover, 358 samples were performed simultaneously by CC, FISH and quantitative RT-PCR. 179 (50%) samples in CCR showed major molecular response (MMoR, defined as a BCR-ABL x100 ratio < 0.1%): 164 (91.6%) were FISH negative and 15 (8.4%) were FISH positive (1.5-10% positive cells). Discussion. We suggest that interphase FISH is a very reliable method of monitoring the CCgR once it has been achieved. The relationship of FISH with molecular response is at least as good as the relationship of CC with molecular response. It remains to be confirmed if the same results can be obtained on peripheral blood cells, that are already widely used for molecular monitoring. Acknowledgements. COFIN 2005, RFO 2005 and 2006, European LeukemiaNet, ALL grants, Fondazione del Monte di Bologna and di Ravenna.

Table.

<table>
<thead>
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<th>Group</th>
<th>No</th>
<th>FISH negative</th>
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<th>FISH 6-10%</th>
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<td>CGR &gt; 20 metaphases</td>
<td>263</td>
<td>209 (79.5%)</td>
<td>44 (16.7%)</td>
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<tr>
<td>CGR &gt; 20 metaphases</td>
<td>102</td>
<td>74 (72.5%)</td>
<td>21 (20.7%)</td>
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<td>3 (2.9%)</td>
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<tr>
<td>PCG &gt; 20 metaphases</td>
<td>50</td>
<td>26 (52%)</td>
<td>0</td>
<td>15 (30%)</td>
<td>9 (19%)</td>
</tr>
<tr>
<td>CGR and MMoR</td>
<td>179</td>
<td>164 (91.6%)</td>
<td>12 (6.7%)</td>
<td>3 (1.7%)</td>
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Although Bcr-Abl represents one of the best characterized oncogenic protein and its role in the pathogenesis of chronic myeloid leukaemia is well established, the molecular mechanisms by which BCR-ABL triggers cell proliferation and transformation remains still partially unknown. Especially, it remains to be elucidated most of the molecules interacting with Bcr-Abl and many of the mechanisms responsible for CML progression. In this study, we set up an approach for genetic analysis investigations to identify a number of candidate genes and pathways involved in disease progression and imatinib resistance based on the Drosophila melanogaster. This approach has the potential to identify genetic pathways that cause the disease, as well as those that can influence it, and does not require a specific knowledge of the function of the disease gene. We generated two different stable transgenic fly lines expressing both human p210bcrr-Abl forms (either wt. or the mutated form T315I) in a tissue specific manner, in particular, the activation of BCR-ABL led to a particular phenotype in the fly eyes. Transgenic flies will be phenotypically and genotipically characterized carefully by analyzing the eye development. We conducted a genetic screening for Bcr-Abl genetic interactors, which allowed the identification of genes encoding proteins acting either as positive or negative regulators in p210Bcr-Abl signal transduction pathways and oncogenesis. All the data obtained with the use of fly model were confirmed in both cell lines and in primary cells via the overexpression and/or silencing of the genes identified with the Drosophila genetic-screening. Finally we have set up and validated a rapid tool for drug testing basing on the observation of the eye phenotype induced by BCR-ABL can be reverted by a complete block of activity induced by the drugs examined. This is easily accomplished feeding flies with food previously mixed with the different drug molecules. With this method we will be able to screen drug libraries to identify molecules able to silence Bcr-AblT315I tyrosine kinase activity. Molecules showing a good inhibitory activity should be quickly identified because their capacity/ability to revert the abnormal eye phenotype displayed by the transgenic flies.

AURORA KINASE INHIBITORS IN THE TREATMENT OF CML AND PH+ ALL PATIENTS RESISTANT TO IMATINIB

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Background. The T315I-8cr-Abl mutation is responsible for about 15% of the cases of relapse in CML and ALL Ph+ patients on imatinib therapy and is also the main mechanism of resistance to the second-generation tyrosine kinase inhibitors. A promising molecular target could be constituted by Aurora kinase, a family of serine/threonine kinases which regulates diverse cell cycle events and is over expressed in a wide variety of solid tumors. The Aurora A isotype contributes to centrosome separation and maturation. Its over-expression causes an increase in centrosomal numbers and aneuploidy, leading to the transformation of mammalian cells. Aurora B kinases are required for bipolar chromosome orientation and condensation. Several substrates for Aurora B have been described, including histone H3 and INCENP. Its over-expression causes loss of kinetochore attachment to microtubules and exit from mitosis. Several compounds have been pre-clinically screened for their activity against Aurora kinases and all of them were active against a variety of human tumor xenograft models. Moreover many of these showed inhibitory effect of T315I-Bcr-Abl with high affinity. Aim of this study was to focus on the role of a new compound, PHA-739558, that selectively inhibits the ATP site of Aurora A and B kinases and has showed, haematologica/the hematology journal | 2007; 92(s3) | 45
in in vitro cancer cell lines, an inhibitory activity on ABL, including the T315I mutant. Our aim is to evaluate the clinical efficacy and the safety profile of the compound, administered to patients affected by CML, relapsing on Gleevec or c-ABL therapy, and to explore the modulation of histone H3 and CRKL phosphorylation. Results. To date, six patients in blastic or accelerated phase of CML, previously unsuccessfully treated with imatinib and other tyrosine kinase inhibitors, have received PHA-739585. The drug has been administered by a weekly 6-hour continuous intravenous infusion, at the dosage of 350 mg/sqm. In some cases, characterized by severe hyperleucocytosis, the schedule of treatment has been modified, either with a biweekly infusion, or with a higher dosage, increased to 400 mg/sqm. In all patients PHA-739585 is showing a relevant hematological activity, in term of reduction of peripheral WBC, especially observed in the first three days following the infusion, and a very low extrahematological toxicity profile. No adverse events related to the drug can be documented. Complete clinical and molecular data will be presented in details in the near future. Acknowledgments. Supported by: European LeukemiaNet, COFIN 2003, AIRC, Fondazione del Monte di Bologna e Ravenna.

CO-104
IMATINIB 400 MG AND PEGYLATED RECOMBINANT INTERFERON-ALPHA IN EARLY CHRONIC PHASE CML. ANALYSIS OF SURVIVAL AND RESPONSES IN THE LONG TERM
Baccarani M,1 Falandrì E,1 Castagnetti F,1 Poirier A,1 Iacobucci L,1 Testoni N,1 Amabile M,1 Marzocchi G,1 Trabacchi E,1 Pungolino E,1 Papineschi E,1 Tiribelli M,1 Intermesoli T,1 Giuliano F,1 Gobbi M,1 Saglio G,1 Pane F,1 Liberati AM,1 Martinielli G,1 Rosti G,1 on behalf of the GIMEMA CML Working Party
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Introduction. To date, imatinib (IM) is the established first line treatment of chronic myeloid leukemia (CML), but for over two decades, patients with CML have been treated with Interferon alpha (IFNalpha), which was the first drug to induce a cytogenetic response. In 2001, 76 early chronic phase (ECP) CML patients (pts) were enrolled in a phase II study of the Italian Cooperative Study Group to investigate the efficacy of IM 400 mg daily and a variable pegIFNalpha dose (50, 100 and 150 microg/wk). Methods. Pts were monitored for conventional cytogenetic and molecular response every 6 months. Peripheral blood samples for quantitative molecular analysis (RT-Q-PCR, Bcr-Abl/Abi x 100 - Taqman) were centralized in Bologna. Major molecular response (MMolR) was defined as BCR-ABL/ABL% less than 0.05. Results. Median observation time is 60 mos (range: 12-67). 75/76 pts discontinued PegIFNalpha, 45 (59%) within 12 months and 21 within the second year, because of absence or loss of the CCgR (8 pts), allogeneic transplantation (1), progression to AP (2), adverse events (4). A complete hematologic response was achieved in all but 2 pts. 66 pts obtained a complete cytogenetic responses (CCgR) in 3 to 42 mos (median: 6), 53 (70%) within 12 mos. 3 pts lost the CCgR, for an overall rate of CCgR of 83% at 60 mos. 54 of the 63 pts with stable CCgR were evaluable for molecular response. The frequency of MMolR was 60%, 94%, 96% at 6, 12 and 24 months, and remained stable at subsequent evaluations. 24 pts (44%) had a stable MMolR while 25 fluctuated between MMolR and a molecular negativity. 2 pts were persistently RT-PCR negative at all observations. 3 pts progressed to AP or BC, 6 pts underwent an alloSCT and 3 pts died (1 after alloSCT, 1 because of disease progression and 1 because of breast cancer). At 60 mos, the estimated rate of PFS and OS were 95% and 96%. Conclusions. The combination of IM with PegIFNalpha allowed a rate of CCgR of 83% at 60 mos and a MMolR rate remarkably high and stable during follow-up. Nonetheless, it resulted in a considerable toxicity, which caused PegIFNalpha discontinuation in all but 1 cases. The question of whether the combination of IM (or of a new tyrosine kinase inhibitor) with lower doses of IFNalpha may provide a definite cure for CML patients remains of great relevance and might be explored in further studies.

Figure 1.
Lymphomas II

CO-105
IS THERE A TUMOR BURDEN LIMIT FOR SUCCESSFUL ABVD CHEMOTHERAPY IN HODGKIN LYMPHOMA (HL)?


Background. The main obstacle to the cure of HL is the resistance to chemotherapy, clinically expressed by incomplete response to first-line therapy (therapeutic failure) or early relapse (within 12 months). The aim of this study was to explore the possibility to identify in advance the patients destined to resist (a target missed so far) in a population of cases treated with ABVD (the current gold standard for HL) and evaluated also through the assessment of the tumor burden volume before therapy (the best pretherapeutic predictor in HL). Materials and Methods. We studied 197 patients treated with ABVD, 4-6 cycles + IF-RT in the 101 stage IA-IIA, 6-8 cycles with optional IF-RT in the 96 stage IIIB-IV cases. All the clinical, laboratory and imaging data recommended for HL were collected. The volume of the total tumor mass was calculated from the slices of the total body staging TC and was normalized to body surface area (relative tumor burden, rTB). The mean follow-up was 45 months (15-149). Events for chemoresistance were considered 13 responses less than good partial remission and 20 early relapses. Splenic involvement was considered together with extranodal lesions. The analysis was made by logistic regression. Results. The rTB was far the best predictor and the only other factor which retained statistically significant power after consideration of rTB was the extranodal (E) involvement. After these two factors all the other conventional prognosticators of HL showed negligible importance. The logistic regression including rTB and E involvement allowed to estimate that the risk of resistance increases by a factor 2 every 100 ccm/sqm rise of rTB and by a factor 3.5 in case of E involvement; moreover, the risk linked to an E lesion corresponds to that of an additional rTB amount of 145 ccm/sqm. A clinically significant threshold limit of rTB seems to be around 250 ccm/sqm, associated with a sixfold increased relative risk (7% of failures < 250 ccm/sqm, 4% over this limit). A nomogram makes this relationship self-explaining and potentially useful for clinical purposes. Conclusions. Though the extranodal lesions were individually measured and their volumes included in the total tumor burden, their prognostic value showed to be beyond their simple volume. But such additional risk linked to E lesions can be expressed in terms of rTB, and a simple graph can express the probability of resistance to ABVD therapy in terms of tumor volumes.

CO-106
IMMUNOPHENOTYPE AND HIGH IPI SCORE AS PROGNOSTICS FACTORS FOR UPFRONT INTENSIFICATION THERAPY IN DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

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The International prognostic index(IPI)is considered to be the most important prognostic score for survival and the strongest indicator for identification of high risk diffuse large B cell lymphoma (DLBCL)patients. Recently,development of DNA techniques as gene expression profiling, have provided more informations about the clinical heterogeneity of DLBCL, trying to match prognosis and molecular features of this particular subset. These results have been translated into a more applicable clinical approach using immunohistochemistry (IHC)through the different expression of CD10, Bcl 6, Bcl 2, IRF4/ Mum1, CD30, CD138 in the tissues. Three distinct patterns allowed to divide the DLBCL in three different subtypes according to cell origin: Germinal center type(GC), activated B cell type (ABC) and not classifiable (NC). We have explored this phase II multicenter study with a risk adapted treatment including, in one arm, autologous stem cell transplantation (ASCT), with the purpose to address a dose intensification only for high risk patients and to avoid overtreatment in the other low risk group. Between September 2004 to April 2007 we have enroled 48 newly diagnosed patients with DLBCL, GC type and non-GC type (ABC type and NC type) with IPI ≥2. The non-GC patients underwent standard R-CHOP chemotherapy for 6 cycles followed by IF (ifosfamide, epirubicin, etoposide) for one cycle, peripheral blood stem cell (PBSC) collection and ASCT conditioned with BEAM. The GC ones were treated with R-CHOP for 6 cycles alone and undergo the ASCT only when in partial response (PR) after R-CHOP. Twelve patients were GC DLBCL, 36 were non-GC. The following Table reports the complete response rate(CR), overall survival rate(OS), the disease free survival rate(DFS) of both subset. Although the small number of patients and the short follow up, our study suggests that the phenotype subdivision in high risk DLBCL treated with immunochemotherapy,might have a little clinical value and that IPI risk appears to be mitigated by upfront intensified therapy with ASCT.

Table.

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>CR rate after R-CHOP</th>
<th>GC patients</th>
<th>non-GC patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>12 months median DFS</td>
<td>76.4%</td>
<td>68.8%</td>
<td>85.4%</td>
</tr>
<tr>
<td>16 months median OS</td>
<td>91.6%</td>
<td>91.0%</td>
<td>89.9%</td>
</tr>
<tr>
<td>16 months median relapse rate</td>
<td>5.0%</td>
<td>5.4%</td>
<td>4.5%</td>
</tr>
</tbody>
</table>

Limited data exist about the role of second-line chemotherapy response assessed by FDG-PET as prognostic factor in aggressive NHL undergoing ASCT. The aim of this study was to investigate the value of pre-transplant FDG-PET for predicting outcome of patients (pts) with aggressive NHL undergoing ASCT. 72 consecutive pts affected by aggressive NHL, either diffuse large B-cell lymphoma (DLBCL) or Follicular Lymphoma grade III (FL), treated at our institution with second-line chemotherapy (IEV regimen) followed by ASCT between September 2002 to September 2006, were considered: median age was 47 years (range 20-67), MVF (44/28), DLBCL/FL (51/21), stage II/III/IV (21/12/39); all pts were evaluated by FDG-PET after 1 (n=54) or 2 (n=18) courses of IEV chemotherapy before ASCT. Pts were categorized into PET negative (Group A= 48) and PET positive (Group B= 24). The ASCT conditioning schedule was the BEAM regimen in all cases. 21 pts underwent IEV followed by ASCT in complete response (CR) after first line chemotherapy, 39 pts were in partial response (PR), 11 pts were relapsed/refractory. Median follow-up time was 24 months (range 6-48). Overall survival rate (OS) of all pts was 81.7%, and the disease free survival (DFS) rate was 67.4%. There was a significant difference in DFS and OS between Group A and Group B: DFS rate for Group A was 84.8% vs 26.7% (p=0.0001; HR 9.37), and of the Group A was 91.8% vs 63.1% for Group B (p=0.033; HR 5.72). There was no difference in outcome between pts who underwent second line chemotherapy and ASCT in CR after first line and pts who obtained CR after second line (PR and relapsed/refractory after first line) (OS 94.4% vs 89.9%, p=0.74; DFS 90.5% vs 88%, p=0.86). There was a trend toward a better OS for FL pts compared to DLBCL: OS of 93.3% vs 78%; the DFS was higher for DLBCL pts compared with FL pts (79.1% vs 61.1%, p=0.14) although the p value was not significant (p=0.22 for OS and p=0.14 for DFS). Bivariate analysis showed that only pre-transplant PET response was an independent survival predictor (p<0.01). These data indicate that pre-transplant FDG-PET has a strong value in predicting the outcome of pts with aggressive NHL undergoing ASCT. Pts with a positive pre-transplant FDG-PET have a 9.3-fold increase in the likelihood of relapse and a 5.7-fold increase in the likelihood of death compared with pts with a negative FDG-PET. Relapse rate of PET positive pts is particularly high (73.5%).
CO-108
GENOMIC INSTABILITY IN THE DEVELOPMENT OF NON-HODGKIN LYMPHOMAS SECONDARY TO HODGKIN LYMPHOMA
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Genomic instability plays a critical role in the process of cancerogenetic secondary to Hodgkin lymphoma (HL). Secondary non-Hodgkin lymphomas (NHL) present additional and potentially distinct aspects compared to solid tumors or leukemias. In fact, both HL and NHL may originate from a common genetically unstable lymphoid progenitor. Indeed, genomic instability required for antibody diversification is a distinctive trait of differentiation processes of B lymphocytes from which HL and most NHL arise. In particular, in the B cell context unfaithful resolution of double strand breaks (DSB) induced by chemo-and/or radiotherapy treatments for HL may promote genomic aberrations that distinguish the transformed components of NHL. Here we report the results of array-based comparative genomic hybridization (aCGH) on lymph node biopsies of 10 HL patients at HL diagnosis and NHL outcome. In HL lymph node biopsies at diagnosis, where the neoplastic component accounts for <2%, aCGH mirror individual genotypic profiles (in preliminary experiments we did not found differences in DNA sequence copy numbers of whole lymph node and peripheral blood mononuclear cells), while in NHL biopsies, where transformed cells account for 80% or more, aCGH would reflect tumor genomic profiles. ACGH performed using DNAs from single HL or NHL lymph node biopsies as tests and pooled DNAs from reactive lymph node biopsies as reference disclosed a great number of genomic imbalances, most likely due individual variability. Statistical analysis (Bonferroni test) let distinguish common genomic imbalances encompassing 15 amplifications and 16 deletions in HL, and 8 amplifications and 7 deletions in NHL. Surprisingly, in all cases genomic profiles of NHL were identical to those of HL. The common genome of lymph node transformed components was further reinforced by the Neighbor Joining Tree analysis in mitochondrial DNA extracted from Reed-Sternberg cells and neoplastic regions in NHL. Differences in the expression levels of gene products involved in DSB resolution (Artemis and DNA-PK) and genomic integrity surveillance (p53 and CHK1/2) in various lymph node components currently under investigation would help to elucidate the pathways involved in genomic instability of B lymphomas.

CO-109
NPM/ALK BUNDLES AND PHOSPHORYLATES THE RNA/DNA BINDING PROTEIN PSF IN ANAPLASTIC LARGE CELL LYMPHOMA
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Anaplastic large cell lymphoma (ALCL) comprises a heterogeneous group of CD30/Ki-1+ T-cell or null-cell lymphoid neoplasms. A subset of ALCL can be characterized by the expression of fusion proteins involving the Anaplastic Lymphoma Kinase (ALK). ALK is a receptor tyrosine kinase normally expressed in specific tissues of the central nervous system during embryogenesis. Due to chromosomal translocations involving the ALK gene at 2p23, ALK is also aberrantly expressed in lymphoid tissues. To date, 11 ALK fusion proteins have been detected in ALCL, the most common of which is nucleophosmin NPM/ALK, occurring in 70% of ALK+ ALCL cases. NPM/ALK, the product of the t(2;5)(p23;q35) translocation, encodes a chimeric 80 kDa protein The oncogenic fusion tyrosine kinase, NPM/ALK, induces cellular transformation (Artemis and DNA-PK) and genomic integrity surveillance (p53 and CHK1/2) in various lymph node components currently under investigation would help to elucidate the pathways involved in genomic instability of B lymphomas.

Figure 1.

The interaction between NPM/ALK and PSF was dependent on an active ALK kinase domain and PSF was found to be tyrosine phosphorylated in NPM/ALK expressing cell lines and in primary ALK+ ALCL samples. Furthermore, PSF was shown to be a direct substrate of purified ALK kinase domain in vitro and PSF Tyr293 was identified as the site of phosphorylation. Y293F PSF was not phosphorylated by NPM/ALK and was not delocalized in NPM/ALK+ cells. The expression of ALK might contribute to its oncogenic transformation. Using a proteomic approach, several RNA/DNA binding proteins were found to co-immunoprecipitate with NPM/ALK, including the multi-functional polypyrimidine tract binding protein-associated splicing factor (PSF) (summarized in Figure 1 A). MS/MS profiles identifying the PSF and NPM/ALK proteins.
fus proteins induced delocalization of PSF from the nucleus to the cytoplasm and forced overexpression of PSF inhibited proliferation and induced apoptosis in cells expressing NPM/ALK. PSF phosphorylation also increased its binding to RNA and decreased the PSF-mediated suppression of GAGE-6 expression. In conclusion, we report here that NPM/ALK associates with and phosphorylates several RNA-DNA binding proteins, in particular the multi-functional nuclear factor PSF. The data also show that PSF delocalizes to the cytoplasm in cells containing constitutively active forms of ALK through a mechanism that involves NPM/ALK-dependent phosphorylation of Tyr295 of PSF. Data were also provided showing that forced PSF expression impairs cell growth, induces apoptosis and decreases clonogenic potential of NPM/ALK-expressing cells. Therefore, PSF might play a role in NPM/ALK-mediated lymphomagenesis through mechanisms that require further investigation. Whether PSF could be a target for other oncogenes also remains to be determined.

Table. Summary of proteins co-immunoprecipitating with NPM/ALK from SUDHL-1 cells, identified by MALDI-TOF. Proteins were identified in 6 independent experiments.

<table>
<thead>
<tr>
<th>Protein</th>
<th>MW (kDa)</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>P01338</td>
<td>77(27)</td>
<td>Nuclear DNA/RNA binding protein</td>
</tr>
<tr>
<td>P23506</td>
<td>94(thoretical)</td>
<td>Cytoplasmic protein responsive to the heat shock family of molecular chaperones</td>
</tr>
<tr>
<td>P09907</td>
<td>90</td>
<td>Cytoplasmic protein binding to the heat shock 90 family of molecular chaperones</td>
</tr>
<tr>
<td>P38078</td>
<td>90</td>
<td>Cytoplasmic protein binding to the heat shock 90 family of molecular chaperones</td>
</tr>
<tr>
<td>P38347</td>
<td>67-65(early)</td>
<td>Nuclear DNA/RNA binding protein involved in pre-mRNA processing, transactivation and DNA repair</td>
</tr>
<tr>
<td>Q01844</td>
<td>68</td>
<td>DNA binding protein involved in oncogenic fusion proteins with various transcription factors</td>
</tr>
<tr>
<td>P11142</td>
<td>64</td>
<td>Heat shock cognate 70 protein</td>
</tr>
<tr>
<td>Q01123</td>
<td>93</td>
<td>Regulatory subunit of the universal protein processing required for the acidification of endosomes and lysosomes</td>
</tr>
<tr>
<td>Q01123</td>
<td>93</td>
<td>Nuclear DNA/RNA binding protein involved in pre-mRNA processing, transactivation and DNA repair</td>
</tr>
</tbody>
</table>

CO-110

FCγRIIA AND FCγRIIA POLYMORPHISMS DO NOT PREDICT THE CLINICAL OUTCOME OF FOLLICULAR NON HODGKIN’S LYMPHOMA PATIENTS TREATED WITH SEQUENTIAL CHOP AND RITUXIMAB

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Introduction. Rituximab mediates its anti-lymphoma effect by different mechanisms of action one of which is being the antibody-dependent cellular cytotoxicity (ADCC) mediated by receptors that recognize the constant region of immunoglobulin (FcR). Polymorphisms of FcγRs at position 131 of FcγRIIA (FcγRIIA-131V/F) and 131 of FcγRIIAll (FcγRIIALL131H/R), have been associated with a better clinical and molecular response in patients treated with Rituximab containing programs. This study was performed to evaluate the role of FcγRs polymorphisms on the clinical outcome of Follicular Lymphoma (FL) patients treated with the sequential administration of CHOP and Rituximab. Methods. In a cohort of 94 newly diagnosed FL-patients treated with a sequential CHOP and Rituximab program, we analyzed FcγRIIA-158V/F and FcγRIIALL131H/R polymorphisms, using a LightCycler platform and sequencing on an ABI Prism 310 Genetic Analyzer. Evaluation of minimal residual disease of Bcl2/IGH cells was also performed by qualitative PCR analysis on BM samples from 68 of 94 patients. Results. The distribution of genotypes in our cases was similar to that previously reported: 32% HH, 49% HR and 19% RR for FcγRIIA and 19%VV, 49%VF and 32% FF for FcγRIIALL. The overall response rate was not different for patients with FcγRIIA-VV or FcγRIIALL-FF (95% vs. 88%, p=ns) and for patients with FcγRIIA-HH or FcγRIIALL-HR (87% vs. 91%, p=ns). The rate of complete remission was similar, irrespective of the different FcγRs variants, being 78% for FcγRIIA-VV, 70% for FcγRIIALL-VF (p=ns), 78% for FcγRIIA-HH and 73% for FcγRIIALL-HR (p=ns). With a median follow-up of 5.8 years, the overall survival at 8 years of this FL cohort was 83%. The EFS of patients with FcγRIIA-FF was 43% compared to 40% in patients bearing the combined FcγRIIA-VF or FcγRIIALL-VF (p=ns) and the EFS of patients with FcγRIIA-HH was 59% compared to 41% for patients with the FcγRIIALL-HR (p=ns). On the contrary, the long-term EFS of patients who achieved FCR negativity for BCL2/IGH at 1 year after the end of CHOP and Rituximab treatment, remains similar irrespective of the EFS of patients who never converted or rapidly lost a molecular response (52% vs. 24%, p=0.0009). Discussion. Although FcγRIIA-158VV and FcγRIIALL-131HH genotypes are predictors of better clinical and molecular response in FL patients treated with Rituximab alone, this association seems to be lost when Rituximab is used with or after chemotherapy.

CO-111

EPIDEMIOLOGIC, CLINICAL AND PATHOLOGICAL FEATURES, RESPONSE TO FIRST-LINE THERAPY AND SURVIVAL OF 151 PATIENTS WITH MYCOSIS FUNGIFORMES

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Mycosis FUNGIFORMES (MF) is a disease with an obscure etiopathogenesis and a great variability of clinical behaviour, well described in the new classification system, the 2005 WHO/EORTC. We aimed to compare the epidemiological characteristics of MF patients with a healthy population in order to highlight any significant difference potentially involved in the pathogenesis of the disease; we also analysed the clinicopathological features of MF patients, in order to highlight those parameters with a prognostic value in the outcome of patients. Patients and Methods. MF patients recluted from 1990 to 2006 were compared to a control group of healthy subjects, matched for sex, age and geographical provenience, selected from the families of patients with other haematologic diseases referred to our Institution. Patients and controls filled out a form with the familiar history for cutaneous disease, immunologic disorders, neoplasms; personal habits (alcohol, smoking); eye and hair color; type of photoexposition, phototype, and reaction to sun-light; past medical history, of previous cutaneous diseases and respiratory diseases and of dramatic disorders, questions, hematologic diseases and neoplasms. Patients’ clinical data (age at first lesion and at diagnosis, type and distance of photoexposition, phototype, and reaction to sun-light; past medical history, of previous cutaneous diseases and respiratory diseases and of dramatic disorders, questions, hematologic diseases and neoplasms). In a cohort of 191 MF patients (128 M, 63 F), with a median age of 65 yrs (range, 18-88). Patients differed from 191 controls (73 M, 118 F, with a median age of 65 yrs, range 17-93) for a higher incidence of cutaneous diseases and neoplasms in the familiar history, of previous cutaneous diseases and respiratory diseases and of
smokers and sensibility to solar exposition in the personal history. In patients, first lesions appeared at a mean age of 56 yrs, with a mean interval to the diagnosis of 67 mo.s. Patches were the most frequent type of lesions (78%), compared to nodules (3%), plaques/papules (16%) and erythroderma (3%). Lesions were disseminated in 65%, multiple with regional distribution in 30% and single in 5%. Pruritus was the most frequent symptom (51%). The most frequent first-line therapy was the combination of phototherapy and interferon (70%); less frequently applied were electrotherapy (12%), mono-/poli-chemotherapy (9%), radiotherapy (0.5%), surgery (0.5%), other therapies (4%). The overall response rate was satisfactory in MF (>90%, with >80% CR). Median time to CR was 5 months in MF. In the follow-up, MF patients experienced a minor event in 30% and a major in 7%. Major-EFS was statistically significantly worse in patients with tumors/erythroderma (median, 18/19 mo.s) than in patients with patches (98% at 186 mo.s) and papules/plaques (80% at 83 mo.s). At last follow-up 19 patients (10%) were dead. OS rate was 83% at 140 mo.s. At the time to relapse was 5 months in MF. In the follow-up, MF patients experienced a minor event in 30% and a major in 7%. Major-EFS was statistically significantly worse in patients with tumors/erythroderma (median, 18/19 mo.s) than in patients with patches (98% at 186 mo.s) and papules/plaques (80% at 83 mo.s). At last follow-up 19 patients (10%) were dead. OS rate was 83% at 140 mo.s. Discussion and conclusions. Our - proliferative disorders of genetic factors responsible for a familiar predisposition to cutaneous disease and tumors. Per- centage, like phototype, previous history of cutaneous and respiratory diseases, seem to be also important. Moreover, our study confirms the prognostic importance of stage in MF. MF with patch/plaque lesions is an indolent disorder with a risk of minor events, whereas MF with tumors/erythroderma behaves aggressively.
CO-113
IN VITRO ACTIVITY OF TYROSINE KINASE, FARENSYL TRANSFERASE AND AKT KINASE INHIBITORS ON C-KIT POSITIVE/NEGATIVE AND MDR-PGP POSITIVE/NEGATIVE LEUKEMIA TUMOR CELL LINES

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IMATINIB is an inhibitor of the tyrosin kinase (TK) activity associated to BCR-ABL, platelet-derived growth factor receptor (PDGFR) alpha and beta and c-kit. Nevertheless, no significant clinical benefit was observed in c-kit positive acute myeloid leukaemia (AML) treated with Imatinib. This may be partially related to the over-expression of some multidrug resistance (MDR) proteins, in particular MDR-Pgp. The toxic effect of a 7-day incubation with increasing doses of TK inhibitors (Imatinib, Nilotinib, Dasatinib), or FTIs inhibitors (Tipifarnib, Lonafarnib) or AKT inhibitor (A-445545) showed a very low growth inhibitory effect. Neither FTIs nor AKT inhibitors were influenced by the MDR-Pgp over-expression. In all the tested combinations we did not measure a synergetic effect, but only an additive effect. The results of our in vitro study indicate that the MDR-Pgp over-expression doesn’t seem to reduce the sensitivity of c-kit positive cells to TK inhibitors, FTIs or AKT inhibitor. The low cytotoxic activity of these molecular target drugs on the tested acute leukaemia a cell lines suggest that c-kit has a role in the pathogenesis of AML. In absence of synergistic effects, the combination of different inhibitors could be more toxic than useful, because the costs in terms of haematological and extra-haematological toxicities could be much higher than the benefits in terms of antitumor activity. Acknowledgments. This work was supported in part by FIRB (protocol number: RBBA01RLNB005 – 2004; D. Russo), progetto 60% 2005 (D.Russo) and COFIN 60% 2006.

CO-114
IN VITRO CHEMOSENSITIVITY TO ASTA-Z OF NORMAL NON LEUKEMIC CFU-GM PREDICTS CD34+ MOBILIZATION IN AML PATIENTS


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Introduction. A proportion of AML patients does not show an efficient mobilization of CD34 cells in P.B. (10-30%), however, no factors predicting mobilization strength in AML patients have been identified so far in studies addressing this issue. Moreover mobilization of an high number of CD34+ cells in P.B. has been associated in patients affected by AML with a poor prognosis. Either a variable pharmacokinetics of chemotherapeutic drugs or, alternatively, an intrinsic chemo-resistance of bone marrow precursors have been hypothesized as possible explanation for the association between amount of residual disease in CR, and therefore prognosis, with strength of CD34+ mobilization, but no data are available to discern between these hypotheses. Methods. In order to answer to this question, in a group of 30 consecutive AML patients in 1st CR, we assessed the in vitro chemosensitivity of normal CFU-GM (obtained in CR) to maphosphaamide and correlated it to CD34+ mobilization peak reached in P.B. All patients were treated according to the same protocol for leukaemia treatment and all were studied in CR at the same time point. Residual CFU-GM growth measured after ASTA-Z incubation was normalized to the baseline value of CFU-GM obtained in absence of the drug. Results. Between residual CFU-GM and peak of CD34+ cells in P.B. during mobilization there was a highly significant correlation (r=0.609, p<0.0003). Normalised residual CFU-GM was the only factor that predicted for CD34 mobilization while no significant correlation was found between maximum peak of CD34+ obtained during mobilization and other patient’s characteristics such as age, body weight, WBC at diagnosis, WBC in CR, FLT, length of aplasia during induction chemotherapy. Patients with a in vitro sensitivity to ASTA-Z below the mean had also a lower number of CD34+ cell in bone marrow harvests (p=0.02). Sensitivity of CFU-GM to ASTA-Z was found to correlate with that observed after in vitro exposure to VP16. Discussion. In conclusions, first: the chemo-sensitivity of normal bone marrow cells in AML is an important determinant of the entity of CD34+ mobilization; second: the intrinsic chemo-sensitivity of normal bone marrow cells is highly variable and it is important in determining the degree of bone marrow damage induced by induction and consolidation chemotherapy.
Improving Outcome in Young Adults with Acute Myeloid Leukemia in First Remission, Undergoing an Allogeneic Bone Marrow Transplant


Background. Acute myeloid leukemia (AML) is a malignant disease characterized by abnormal proliferation of clonal precursor cells. Although different strategies have been performed to obtain complete remission, disease progression actually occurs in about 60-70% of patients. Therefore, new alternative strategies are urgently required. The proteasome inhibitor Bortezomib has a documented antitumor activity in multiple myeloma and other lymphoid malignancies. Tumor Necrosis Factor Related Apoptosis Induced Ligand (TRAIL) is a member of the TNF family that induces apoptosis in tumor cells while sparing normal cells. Here we evaluated the sensitivity to Bortezomib alone or in combination with TRAIL.

CO-118
67 KDA LAMININ RECEPTOR (67LR) EXPRESSION AND FUNCTION IN NORMAL AND ACUTE MYELOID LEUKEMIA CD34+ CELLS


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Introduction. We have recently documented a strong upregulation of 67 KDa laminin receptor (67LR) in granulocyte colony stimulating factor (G-CSF)-mobilized CD34+ cells. This receptor, which is expressed on hematopoietic stem cell (HSC) egress from bone marrow (BM) into the circulation. We are now investigating 67LR expression and function in CD34+ acute myeloid leukemia (AML) cells. Methods. BM specimens were obtained from healthy donors or from AML patients. Enumeration of CD34+ cells in BM mononuclear cells (MNC) were performed by 3-color flow cytometry on a MNC gate of CD45- cells. Cell adhesion assays were performed in 96-well plates coated with laminin (10 μg/mL). Cell migration assays were performed in Boyden chambers, uncoated or coated with laminin, stromal derived factor 1 (SDF1) or serum-free medium, used as chemoattractants. Results. Flow cytometric analysis of BM-MNCs from 20 normal subjects and from 20 CD34+ AML patients showed that normal BM CD34+ cells express very low levels of 67LR (mean: 5.1%), whereas 60% of CD34+ AMLs express high levels of 67LR. Therefore, 67LR expression is lower in normal BM CD34+ cells in steady-state conditions, whereas it is strongly upregulated in BM CD34+ AML cells, suggesting that increased 67LR expression could confer to leukemic CD34+ cells a migratory phenotype, as already demonstrated in G-CSF-stimulated normal CD34+ cells and in metastatic cancer cells. We also investigated in vitro adhesion and chemotaxis assays whether 67LR upregulation could be responsible for increased laminin-dependent and SDF1-dependent adhesion and migration of malignant CD34+ HSC. CD34+ cells from the leukemia KG1 cell line were transfected with the 67LR cDNA. 67LR-transfected KG1 cells showed increased migration toward laminin, as compared to untransfected cells; preincubation with a polyclonal anti-67LR antibody strongly reduced their migratory response to laminin. On the contrary, 67LR overexpression did not increase KG1 cell adhesion to laminin, demonstrating that 67LR is mainly involved in mediating CD34+ cell migration to laminin rather than adhesion. Moreover, 67LR-transfected KG1 cells, also showed increased migration toward SDF1. Discussion. Our data document that leukemic HSCs undergo changes in their adhesive properties that affect the retention from BM, and that 67LR overexpression could be responsible, at least in part, for their tendency to migrate into the circulation and to develop extramedullary disease.

CO-119
CYTOTOXIC ACTIVITY OF BORTEZOMIB ALONE AND IN COMBINATION WITH TRAIL IN HUMAN ACUTE MYELOID LEUKEMIA


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Acute myeloid leukemia (AML) is a malignant disease characterized by abnormal proliferation of clonal precursor cells. Although different strategies have been performed to obtain complete remission, disease progression actually occurs in about 60-70% of patients. Therefore, new alternative strategies are urgently required. The proteasome inhibitor Bortezomib has a documented antitumor activity in multiple myeloma and other lymphoid malignancies. Tumor Necrosis Factor Related Apoptosis Induced Ligand (TRAIL) is a member of the TNF family that induces apoptosis in tumor cells while sparing normal cells. Here we examined the sensitivity to Bortezomib alone or in combination with
TRAIL of bone marrow cells from newly diagnosed or relapsed/refractory AML patients (34 patients: 25 newly diagnosed, 4 relapsed, 5 refractory patients). Immunohistochemistry or immunofluorescence using a monoclonal mouse anti-human p65 (Rel A) showed that localization of NF-κB was in the nucleus of AML blasts and do not translocate after Bortezomib exposure. In each sample Bortezomib was able to induce cell death of AML blasts. The antitumor effect was dose and time-dependent (concentration of Bortezomib ranging from 0.001 to 10 micromolar and Mcl-1, upregulation of TRAIL-R1, TRAIL-R2 and p21 and activation of executioner caspases. Moreover, low doses of Bortezomib were able to prime TRAIL-resistant AML cells for enhanced TRAIL-mediated killing. Thus, the combination of proteasome inhibitors and TRAIL is effective for treatment of AML patients even though refractory to conventional chemotherapy.

CO-120
THERAPEUTIC OPTIONS FOR RELAPSED ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A SURVEY FROM THE GIMEMA AL WP (GRUPPO ITALIANO MALATTIE EMATOLOGICHE DELL’ADULTO)
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Introduction. The percentage of long-term survival in elderly patients with acute myeloid leukemia (AML) does not exceed 10-15% due to relatively low complete remission (CR) rate and high incidence of relapse. Besides, few data are available regarding therapeutic options for elderly relapsed AML patients. Methods. Data were collected through a questionnaire e-mailed to 32 hematologic institutions cooperating with GIMEMA Foundation. Questions to be addressed regarded: 1) percentage of relapsed elderly patients treated with aggressive salvage chemotherapy; 2) specific treatment administered; 3) selection criteria for intensive salvage. Results. The percentage of patients enrolled into aggressive salvage regimens varied from 10 to 80% (median 50%) at different Institutions. The most frequent factor influencing the therapeutic choice was performance status (97%). Additional factors were age > 70 years (44%) and duration of first complete remission (53%). Some Centers considered the following characteristics as single factors: concomitant disease requiring specific treatment, cytogenetics at diagnosis, presence of active infections, informed consent and concomitant extramedullary relapse. Regimens including Fludarabine and intermediate dose cytarabine (ARA-C) were most frequently used as aggressive salvage therapy (59%), while Gemtuzumab Ozogamicin (GO) was used in various combinations at 11 out of 32 Institutions (34%). Of note, GO, which is currently registered in Italy for the treatment of AML in relapse, was adopted as a single agent at one Center only. For patients not eligible to aggressive therapy, the most frequent approach included hydroxyurea (59%). Low dose ARA-C (LDARA-C) were adopted at 5 centers either as single agent or in combination. Discussion. The therapeutic approach for elderly AML relapsed patients is extremely heterogeneous within different GIMEMA centers. A marked selection, ranging from 10 to 80%, is operated as to inclusion into aggressive salvage regimens and only a small minority of patients are offered experimental approaches. Work is currently in progress within the GIMEMA group in order to design clinical trials specifically dedicated to the management of this category of patients.

QUALITY OF LIFE - SUPPORT THERAPY - TRANSFUSION MEDICINE

CO-121
HOME CARE SERVICES IN HAEMATOLOGY: A NEW COST ANALYSIS APPROACH
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Introduction. Over the last 15 years the traditional hospital-driven paradigm of care delivery has been challenged by the need to satisfy increasing demand volume and complexity with limited resources. New service concepts, like home care, have been introduced to avoid unnecessary admissions and improve appropriateness. Their cost profile, however, is yet to be established and the question as to whether better care can be more efficiently provided outside the hospital is still unanswered. In 2002 a program to provide high-specialty home care services (HCS) to onco-haematological patients was undertaken by the Dept. of Haematology at Niguarda Hospital, Milan. Along with clinical activity, an economic analysis was carried out in the last 2 years with the aim to assess cost incurred to afford effective HCS. Both provider and societal perspective were adopted in order to disclose cost-shifting from the NHS to patient’s family. Study design. Data from 110 haematological patients enrolled between January 2005 and December 2006 were analysed. Total and daily cost per patient were calculated based on analytical accountability by cost centre and compared with historical data from haematological inpatients undergoing either a standard hospitalization or a day-hospital schedule. Average hospital stay within disease groups was also calculated to weight comparisons with HCS patients by case severity. The impact of therapeutic options (transfusions, chemotherapy, etc.) on HCS cost structure was also assessed. Statistics were obtained by covariance analysis and factor analysis with principal components. Discussion. Available information on cost comparisons of HCS and hospital-based services are biased by the current reimbursement system, where DRG tariffs are not a reliable proxy of hospital costs. Our data in onco-haematology do not support HCS as a cheaper alternative for hospitalization, being mostly aimed at medical and social support in aftercare and palliative care. Savings are rather foreseen when inappropriate admissions can be prevented by a satisfactory long-term patient-services relation. Finally, treatment-related costs only accounted for about 17% of resources spent for patient stay, as compared to 83% incurred for staff and hotel costs, so that the impact of patient severity is almost negligible in our setting. The opposite is true in HCS, where the amount of care provided is a relevant cost-driver, thus supporting a more favourable cost-value profile.

CO-122
EPIDEMIOLOGICAL FEATURES OF PAIN IN HOSPITALIZED PATIENTS WITH BLOOD DISORDERS: AN ITALIAN SURVEY
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Some recently reported experience claimed that the occurrence of pain in haematological diseases may be potentially high. In order to investigate the epidemiology of pain in this setting, a 6-months multicenter study involving four Italian haematological Centres was performed. Pain, as fifth vital sign, was regularly evaluated. A treatment protocol based on the cancer pain WHO analgesic was applied. The study included 421 patients (201 male) with a median age of 67 (17-89) years. Haematological diagnoses (Table 1) were as following: 141 (35%) non-Hodgkin’s lymphomas (NHL), 74 (18%) acute myeloblastic leukaemia (AML), 43 (10%) multiple myeloma (MM), 23 (5%) acute lymphoblastic leukaemia (ALL), 35 (8%) other lymphoproliferative disorders, 35 (9%) myelodysplastic and chronic myeloproliferative disorders, and 71 (17%) non-malignant haematological diseases. Of 421 patients, 157 (37%) experienced almost one pain syndromes. The highest incidences of pain were recorded in MM and in acute leukaemia (77% and 43% respectively); lower values were recorded in other diseases. Among the 157 patients
Chemotherapy. Table 1. Pain in haematological wards: incidence and relative distribution of pain syndromes in haematological diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Total Patients (%)</th>
<th>Pain Patients / Total Patients (%)</th>
<th>No of Pain syndromes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHL</td>
<td>141 (33)</td>
<td>52/141 (37)</td>
<td>82 (37)</td>
</tr>
<tr>
<td>AML</td>
<td>74 (18)</td>
<td>32/74 (43)</td>
<td>45 (20)</td>
</tr>
<tr>
<td>MM</td>
<td>43 (10)</td>
<td>33/43 (77)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>ALL</td>
<td>23 (5)</td>
<td>10/23 (43)</td>
<td>19 (8)</td>
</tr>
<tr>
<td>LPD</td>
<td>34 (8)</td>
<td>34/34 (23)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>MPO</td>
<td>36 (9)</td>
<td>12/36 (34)</td>
<td>15 (7)</td>
</tr>
<tr>
<td>NMHD</td>
<td>71 (17)</td>
<td>10/77 (13)</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>421 (100)</td>
<td>157/233 (37)</td>
<td>223 (50)</td>
</tr>
</tbody>
</table>

NHL: non Hodgkin lymphomas; AML: acute myeloid leucemias; MM: multiple myeloma; ALL: acute lymphoblastic leukaemias; LPD: other chronic lymphoproliferative disorders; MPO: myeloproliferative disorders; NMHD: non-malignant haematological diseases.

Discussion. Table 1. Home Care in Hematology in Rome: some activity and clinical outcome indicators.

<table>
<thead>
<tr>
<th>Activity and Outcome Indicators</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Patients</td>
<td>1782</td>
</tr>
<tr>
<td>Chronic Patients</td>
<td>677 (38%)</td>
</tr>
<tr>
<td>Advanced Patients (100%)</td>
<td>834 (47%)</td>
</tr>
<tr>
<td>Patients on causal treatments (Early discharged)</td>
<td>273 (15%)</td>
</tr>
<tr>
<td>Median days (range) HC duration (Total)</td>
<td>27 (1–2490)</td>
</tr>
<tr>
<td>Median days (range) HC duration (Chronic)</td>
<td>365 (1–2490)</td>
</tr>
<tr>
<td>Median days (range) HC duration (Advanced)</td>
<td>18 (1–407)</td>
</tr>
<tr>
<td>Median days (range) HC duration (Early discharged)</td>
<td>11 (1–826)</td>
</tr>
<tr>
<td>Red Blood Cells Packets</td>
<td>11213</td>
</tr>
<tr>
<td>Platelet Transfusions</td>
<td>1939</td>
</tr>
<tr>
<td>Anti-infectious drugs intravenous infusions</td>
<td>9215</td>
</tr>
<tr>
<td>Patients died (total)</td>
<td>1443</td>
</tr>
<tr>
<td>Patients died at home</td>
<td>1075 (74%)</td>
</tr>
<tr>
<td>Patients died in hospital</td>
<td>368 (20%)</td>
</tr>
</tbody>
</table>

Diagnosis
- Acute Leukaemias: 38
- Lymphomas: 28
- Multiple Myeloma: 13
- Myeloproliferative Syndromes: 13
- Others: 8

Incidence of major symptoms and complications
- Diarrhoea: 9%
- Fatigue: 89
- Pain: 55
- Infections: 49
- Bleeding: 27

Transfusions and intravenous therapy (antibiotic, antiviral, antifungal drugs, fluids and so on) were the major type of domiciliary interventions; analgesic treatments, supportive and rehabilitation therapies and other symptoms control measures were also delivered at home. The cumulative median HC duration for the 1782 patients was 27 days (1-2490) with wide variations due to the different care systems and decision-making models that have been adopted according to life expectancy and phase of the disease. Patients on causal treatments, including those at high risk of complications due to the severe neutropenia or

CO-124

LONG TERM ACTIVITY AND ONGOING DEVELOPMENTS TOWARDS A NEW MODEL OF HOME CARE ORGANIZATION IN ROME. THE ROMAL - GIUSEPPE PAPA HOME HAEMATOLOGICAL HOSPITAL

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Caretucl developments have been achieved in recent years in the field of haematological home care (HC), which has affirmed itself as a safe and effective alternative to the hospitalisation. Most HC haematological services have been traditionally provided by non-profit health care organisations (NPHCOs); however, the integration of such private service with public health institutions has been rarely provided. In Rome, haematological HC services, belonging to the S.Eugenio Hospital and the Human Biotechnology and Haematology Department of La Sapienza University, were started in 1990 and 1993 respectively. As March 2017, 1782 patients with a median age of 70 years (4-98) have been followed at home by the two HC teams. The most relevant findings of their activities are summarised in Table 1.

Table 1. Home Care in Hematology in Rome: some activity and clinical outcome indicators.
Delayed transfusion reactions (DHTRs) are defined as those occurring more than 24 hours following a transfusion of blood or blood components: these are usually delayed haemolytic reactions due to the development of red cell alloantibodies (alloAb). DHTRs occur regularly worldwide despite the fact that it is now general practice to take all red cell alloAb ever detected in a patient’s blood in to account at the time of the new transfusion. With the aim to identify DHTRs and their clinical impact we evaluated 46 patients, 22 male, 24 female, ages ranged from 27 to 91, transfused with red blood cell concentrates (RBC) matched with an extended phenotype and leukodepleted bedside. 14 patients were affected by non haematological disorders (3 anemia in renal failure, 11 chronic iron deficiency). 32 patients were affected by haematological diseases: 15 myelodysplastic syndrome (MDS) 12 myeloproliferative disorders (MPD) 5 thalassemia syndromes (TS). Indirect liss antigenoglobin test (IAT) and direct antiglobulin test (DAT) were performed using a sensitive gel test (DiaMed); an eluate from the red cells was investigated for positive DAT. In non-haematological group (RBC transfused ranged from 4 to 240, mean 56) none developed alloAb, there was a single positive DAT IgG with neither serological evidence of hemolysis nor detectable antibodies in eluate. In haematological group (RBC transfused ranged from 4 to 418, mean 131), 5MDS patients and 2MPD patients had known alloAb (2 anti-K, 1 anti-Lea, 1 anti-c-e, 1 anti-b); 5MDS (20%), 5MPD (42%), 3TS (60%) patients developed a positive DAT IgG. 1MDS, 5TS but none MPD DAT-positive patients had serological evidence of hemolysis (raised plasma bilirubin and LDH, low haptoglobin). Autoantibodies were detected in eluate made from 2 MPD and 1 MDS patients’ red cells; no alloAb were detected in eluates. The policy of testing patients for the formation of new alloAb before every transfusion, to take all red cell alloAb ever detected in to account at the time of the new transfusion and to extending phenocompatibility to non-D Rhesus and Kell system seems to be sufficient to limit DHTRs. May be that DAT positive in some haematological patients (most MPD) depend on damage of red cell membrane with no real clinical impact. In TS patients in which we found DAT positive and haemolyisis but no antibodies were detectable, more sensitive technique will be used to differentiate autoimmune haemolytic anaemia and DHTRs.
QUALITY OF LIFE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Treatment of acute myeloid leukemia (AML) in the elderly is generally tailored on the basis of age, performance status, concomitant diseases and patient consent. Toxicity and low response rates are major constraints and therapeutic options are often conditioned by clinicians opinions rather than patient preferences. Health-related quality of life (HRQoL) may be useful in this setting. We designed a prospective multicenter study to evaluate the predictive potentials of HRQoL measures on prognosis in elderly (> 60 years) AML patients. All consecutive patients admitted to Hospital with de novo AML and capable of completing the QoL questionnaires were included in the study. HRQoL was measured by the QOL-E and the EORTC QLC-C30 questionnaires. One hundred and seven patients (M 55 and F 52) were included in the study. Mean age was 72±6 years. Only 10 patients had an ECOG PS > 1 at diagnosis. The questionnaires showed good internal consistencies. Scores were low at baseline (reflecting poor HRQoL), particularly in the global and QOL-E fatigue and disease-specific scores. The presence of concomitant diseases was associated with poorer QoL. ECOG PS grades were associated with peripheral blasts (p=0.034) and QOL-E functional (p=0.001) and fatigue scores (p=0.02). Baseline Hb levels correlated with QLC-C30 physical functional (p=0.005), role function (p=0.02) and fatigue (p=0.0001). Increasing age was correlated with EORTC physical function (QLC-C30 r=-0.217, p=0.001; QOL-E r=-0.176, p=0.016), role function (QLC-C30 r=-0.245, p=0.001; QOL-E r=-235, p=0.002) and fatigue (QLC-C30 r=0.239, p=0.001; QOL-E r=-0.210, p=0.003) scores. After 1 month, 72 patients were evaluable: a decrease in QOL-E physical functioning (delta=-8, p=0.009), QLC-C30 role functional (delta=-15, p=0.002), QOL-E social function (delta=-10, p=0.012) and an increase in fatigue (QLC-C30 delta=8, p=0.026) were demonstrated. After 6 months, 19 survivors had a significant increase in QOL-E total (p=0.18), functional (p=0.042), specific (p=0.004) and treatment-outcome index scores (p=0.032) and had better initial QoL scores. In elderly AML patients, HRQoL is poor at diagnosis, especially with increasing age, especially in patients with concomitant diseases, and it deteriorates during initial treatment. However, survivors at 6 months experience improvements in HRQoL, representing patients worth treating. HRQoL may indicate patient-tailored therapy in elderly AML patients.

PALIFERMIN REDUCES INTESTINAL TOXICITY AND LENGTH OF HOSPITALIZATION IN MULTIPLE MYELOMA PATIENTS RECEIVING HIGH DOSE MELPHALAN


Introduction. Palifermin has been studied after TBI containing regimen and after BEAM schedule while benefits of this agent has not studied after High Dose Melphalan. Methods. We have employed Palifermin in 20 patients affected with Multiple Myeloma that received high Dose chemotherapy with Melphalan (200 mg/m2) and we compared clinical results obtained using Palifermin with those of a second group of 31 patients affected with MM and treated previously in our Institution using the same High Dose Schedule and the same anti-infectious prophylaxis. The two groups were not different in age (p=0.49), sex (p=0.2), WBC in P.B (p=0.3), and number of lines of chemotherapy received previously (p=0.4). All patients were transplanted using PBSC as hematopoietic rescue and CD34+ dose was not different in the two groups (p=0.9), all patients received G-CSF during aplasia post chemotherapy. Oral mucositis grade was assessed using DMS score. Results. Time for hematopoietic reconstitution and transfusion needs were not different in the two groups. We have not detected significant difference in maximum mucositis score in the two groups, neither duration of oral mucositis resulted different, however Palifermin was associated with a reduction of duration of diarrhea (5 days versus 7 days, p=0.06), with a reduction of use of Total Parenteral Nutrition (p=0.0001) and with a reduction of duration of TPN (15 days versus 43, p<0.0002). Length of hospitalization was also significantly reduced with Palifermin in respect to patients that did not received KGF (11 days versus 16 days, p=0.0006). FDU was diagnosed in only 16% of Palifermin patients versus 34% of group NO-Palifermin (p=0.1). Discussion. In conclusion Palifermin in MM patients undergoing Autologous PBSC transplantation determines a reduction of intestinal toxicity and a reduction of hospitalization length.
PO-001

A PHASE II TRIAL OF FM (FLUDARABINE AND MITOXANTRONE) CHEMOTHERAPY FOLLOWED BY YTTRIUM 90 (90Y) IBRITUMOMAB TIUXETAN (ZEVALIN) PREVIOUSLY UNTREATED INDOLENT NON FOLLICULAR LYMPHOMA PATIENTS


Institute of Hematology and Medical Oncology L. & A. Seràgnoli, University of Bologna; Nuclear Medicine Division, S.Orsola-Malpighi Hospital Bologna; Hematology S. Eugenio, Roma; Hematology ASO S.Giovanni Battista, Torino, Italy

We conducted a prospective, single arm, open-label, non-randomized, multicenter, phase II trial to evaluate the efficacy and safety of 90Y Ibritumomab Tiuxetan (90Y-IT) of a novel new approach combining induction chemotherapy with Fludarabine and Mitoxantrone (FM) followed by consolidation with 90Y-IT for patients with previously untreated indolent non-follicular lymphoma (indolent non-FL). Patient’s eligibility was represented by: patient age 18 years or older with biopsy-proven, untreated, bidimensionally measurable, stage II, stage III, or stage IV indolent non-FL expressing the CD20 antigen; WHO performance status of 0 to 2. Patients were treated with standard FM chemotherapy every 28 days for 6 cycles. Patients were restaged 4 to 8 weeks after completion of one cycle of FM chemotherapy. Patients achieving at least a partial response after 6 cycles of FM chemotherapy were eligible for consolidation with 90Y-IT provided the granulocyte count was greater than 1500/microl, the platelet count exceeded 100.000/microl, and the bone marrow examination at the completion of FM chemotherapy demonstrated no more than 25% involvement with lymphoma. All patients were eligible to receive a single dose of 90Y-IT 14.8 MBC/kg (0.4 mCi/kg). At data of this abstract, 26 patients were enrolled and among these 26 patients 20 were subsequently treated with 90Y-IT. Historically, 10 had marginal zone lymphoma, 8 had lymphoplasmacytic lymphoma, and 8 had a small lymphocytic lymphoma; 13 were male and 13 female; the median age was 61 years (range 45-82); 4 were stage III, and 22 stage IV. After the FM treatment the overall response rate was 80% including 50% CR and 30% PR. Time to event analyses, including TTP and duration of response are pending further follow-up. Treatment was well tolerated; grade 3-4 AEs were seen in 15 patients; the most common grade 3-4 AEs were neutropenia. Among the 20 patients subsequently treated with 90Y-IT (7,2%) patients improved their remission status from PR to CR. The 90Y-IT toxicity included grade 3-4 hematologic AEs in 16 patients; the most common grade 3-4 AEs were neutropenia (11 patients) and thrombocytopenia (16 patients). Red blood cells and/or platelets transfusions were given to 6 patients. These preliminary data represent a: patient age 18 years or older with biopsy-proven, untreated indolent non-FL. The feasibility, tolerability, and efficacy of FM plus 90Y-IT regimen for patients with untreated indolent non-FL.

PO-002

SURGICAL RESTAGING OF PET POSITIVE RELAPSES OF MALIGNANT LYMPHOMA


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In the last few years, fluorodeoxyglucose (FDG)-positron emission tomography (PET) has shown a number of potential advantages in refining and improving the management of Hodgkin’s disease (HD) and aggressive non-Hodgkin’s lymphoma (NHL). PET plays a significant role in the initial staging, in the evaluation of residual masses after therapy, and in the monitoring of therapy response early in the course of the treatment regimen. We reviewed the clinical and pathological data of 30 consecutive patients (21 HD and 9 NHL) with a suspicion of lymphoma relapse on the basis of a positive mediastinal PET scanning. In our experience, PET activity in the mediastinum of a patient being followed up for a mediastinal lymphoma should not be considered sufficient for diagnostic purposes (false positive rate. 48%). Histological confirmation can safely be carried out with various biopsy techniques which include percutaneous core needle biopsy under CT guidance through a 15 G Menghini needle, video-mediastinoscopy, pre-vascular mediastinoscopy, extended video-mediastinoscopy, anterior mediastinotomy, cervicalotomy + manubriotomy (sternal split), video-thoracoscopy (VATS) and standard thoracotomy. A reliable histological confirmation can be obtained in this setting mainly with mini-invasive approaches and low morbidity, provided that the timing and the type of biopsy technique is chosen appropriately, taking into account the clinical and imaging findings of the individual patient.

PO-003

ANALYSIS OF GENETIC POLYMORPHISMS OF METABOLIZING AND DNA REPAIR GENES IN NON-HODGKIN’S LYMPHOMA


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Introduction. Everyone has a unique combination of single nucleotide polymorphisms (SNPs) that modify susceptibility and response to drugs, environmental and carcinogenic exposures. Genetic polymorphisms in the genes encoding for drug-metabolizing enzymes, underlying the variation in enzyme activity, can modify individual susceptibility to cancers as well as the response to therapy. Moreover, it has been hypothesized in many studies that polymorphisms in DNA repair genes reduce their capacity to repair DNA damage and thereby lead to a greater susceptibility to cancer or age-related diseases. To assess whether the individual polymorphic trait may be associated with increased risk of development of lymphoma we screened different common polymorphisms in a series of 65 patients affected by non-Hodgkin’s lymphoma (NHL). We tested polymorphisms of some genes involved in toxicant metabolism and DNA repair. We also analyzed 74 unrelated controls for metabolizing genes; instead for DNA repair gene we referred to control allelic frequency reported in literature. Methods. A total of 15 allelic variants were evaluated, 8 for the metabolizing genes and the 5 for the DNA repair genes: null genotype for GST-M1 and T1, three SNPs for CYP2A4 (-392A>G, Phe189Ser and Leu293Pro), two for CYF2E1 (-1053C>T and -1293G>C), one for for NQO1 (Pro187C>T and -1293G>C), one for for XRCC1 (Arg194Trp, Arg280His and Arg399Gln), one for XRCC3 (Thr241Met) and one for XPD (Gly313Arg). Sequences and selected polymorphisms were retrieved from NCBI dbSNP database. Results. The analyzed NHL patients showed an incidence of polymorphisms in the majority of metabolizing enzymes tested similar to that reported in control groups. In particular we found 4,9% patients carrying the CYF2A4 -392A>G variant, none CYP2A4 -1293G>C, 11,6% CYF2E1 -1053C>T and XRCC3-241 (68,9%) and XPD-751 (47,8%) variants. The prevalence of each polymorphism varies greatly among different ethnic groups and can influence the power and interpretation of epidemiological data, study of more numerous and homogeneous patient/control population is needed.
PO-004
VEBEP AND LOW-DOSE RADIOTHERAPY: A VINORELBINE-CONTAINING THERAPY FOR NEWLY DIAGNOSED ADVANCED HODGKIN'S LYMPHOMA

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Introduction. To test the efficacy and toxicity of a new-generation, vinorelbine-containing, VEBEP regimen in Hodgkin's lymphoma (HL) with low-doses radiotherapy (RT) with the primary aim to reduce short and long-term toxicity and, if possible, to improve therapeutic outcomes. Method. Twenty-four consecutive adult patients with newly diagnosed biopsy-proven HL, classified as stage II A, II B, III (A and B), and IV (A and B) according to the Ann Arbor criteria, were enrolled into this prospective nonrandomized study. The regimen consisted in epipodophyllotoxin 30 mg/mq iv day 1-3, cyclophosphamide 1000 mg/mq iv on day 1, VNR 25 mg/mq iv on day 2, bleomycin 10 mg/mq iv on day 3, and prednisone 100 mg iv day 1-4. Treatment plan varied on the basis of Ann Arbor/Cotswold stage: locally extensive disease were given four courses of VEBEP and involved field (IF) RT at same doses, whereas advanced stages were given six courses of VEBEP with RT only on bulky sites. Results. A total of 105 patients (87%) entered complete response (CR) at the end of the treatment. CR rate was significantly lower in patients with stage IV compared with patients with stage II and III (67% vs 92% vs 92, p=0.004) and in patients with B-symptoms (p=0.02). Toxicity was globally mild, with neither toxicity deaths or hospitalisation. Eighteen (17%) out of 105 complete responders showed lymphoma relapse within six years from the starting chemotherapy. With a median follow-up of 57 months, 72% patients were free from lymphoma progression (FFP). FFP was significant inferior in patients with stage III and IV compared with patients with locally extensive disease (65% vs 78% p=0.009) and in patients with B-symptoms (62% vs 84% p=0.01), respectively. A total of 10 patients have died with disease progression and one of second tumor, for an overall survival rate of 91%. Among the 111 patients alive, all but one are disease free, 87 in first CR, and 23 in second or further CR with an FFP of 74% at 31 months from relapse. Conclusions. Despite VEBEP regimen show a FFP probability lower than other common new regimens, the very low toxicity allows a full salvage therapy with an optimal FFP. An increase in dose-intensity is planned for patients at higher risk.

PO-005
LOW-DOSE ORAL FLUDARABINE PLUS CYCLOPHOSPHAMIDE AS FIRST-LINE TREATMENT IN ELDERLY PATIENTS WITH INDOLENT NON HODGKIN LYMPHOMA

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Fludarabine (Flu) containing regimens have been reported to be highly effective in the treatment of indolent lymphoid malignancies, however excessive toxicity, such as severe infections and myelosuppression occur frequently and particularly in elderly patients. We previously showed that low-dose combination of fludarabine and cyclophosphamide (Cy) resulted in a mild toxicity, still allowing a high response rate both in untreated and relapsed/refractory patients with chronic lymphoproliferative disorders. On the basis of these results we applied this schedule to untreated advanced stage indolent non Hodgkin lymphoma (NHL). Twenty-five elderly patients (11 males and 14 females) with NHL: 6 patient with small lymphocytic lymphoma (SLL), 15 with marginal zone lymphoma (MZL) and 4 with follicular lymphoma (FL), were enrolled in this study. The median age of patients was 74 years. Treatment schedule consisted of oral Flu 25 mg/m²/day (40 mg total dose) and Cy 150 mg/m²/day, both for 4 consecutive days. Treatment was repeated every 28 days for 4 cycles. All patients were evaluable for response and toxicity. Twenty-one out 25 patients (84%) were responsive. In particular, CR was obtained in 10 patients (3 SLL, 5 MZL, 2 FL) while PR was obtained in 11 patients (4 SLL, 5 MZL, 2 FL). The remaining 3 patients (3 MZL and 1 SLL) were considered non-responders and shifted to alternate therapies. During an observation period of 37 months we recorded an overall survival (OS) rate of 70% (median not reached at 37 months) and a median event free survival (EFS) of 20 months. Haematological toxicity consisted mainly of grade 2-3 neutropenia and anaemia (26%), while extra-haematological toxicity was represented mostly by grade 1-2 episodes. Five patients died (20%), four due to progressive disease and one of sudden death unrelated to treatment. This reduced-dose Flu-based oral regimen showed, compared with standard-dose schedules, a lower toxicity with equivalent efficacy. The treatment is easy to administer on an outpatient basis and its tolerability is good.

PO-006
INTENSIFIED FULL DOSE INDUCTION THERAPY (HD-MACHOP-R) AND ASCT AS FRONT LINE THERAPY FOR PATIENTS WITH HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA (B-DLCL)


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Introduction. Despite the addition of Rituximab to CHOP chemotherapy have improved the outcome of patients with B-DLCL, in a large part of cases with high-intermediate risk (HI) and high (H) risk, according to the International Prognostic Index (IPI), the prognosis remain dismal. Post induction consolidation with high-dose treatment (HDIT) and autologous stem cell transplantation (ASCT) have been proposed as a perspective way to improve the rate of response and survival; however the results of different randomized studies have yielded conflicting results. The aim of the present single-centre study was to investigate the therapeutic impact of an intensified induction regimen followed by ASCT in a cohort of previously untreated, HIV negative, adult patients (age 18-60 years) with a histological confirmed diagnosis of B-DLCL. Methods. Twenty-four unselected consecutive patients, median age 41 years, 16 with HI and 8 with H IPI risk were enrolled into the study between June 2002 and April 2006. The therapeutic program was based on the administration of an intensified CHOP-R induction regimen, named HD-MACHOP-R, involved-field radiotherapy on localized residual disease, HDIT and ASCT. The HD-MACHOP-R regimen consisted of two arms (A and B) which were alternated every 21 days for 6 courses. Arm A differentiated from standard CHOP-R because of the addition of mitoxantrone 1800 mg/m² and cytosine arabinoside 1000 mg/m². Arm B differentiated because of the addition of mitoxantrone 500 mg/m², cytosine arabinoside 4000 mg/m² (subdivided in 2 doses) and for the use of iophosphamide 1600 mg/m² (subdivided in 2 doses) instead of cyclophosphamide. Stem cells (SC) harvesting was performed from peripheral blood after the fourth, fifth or sixth HD-MACHOP-R course after G-CSF priming. HDIT was performed between 2 to 3 months after the end of the induction therapy using BEACH as a conditioning regimen. Results. After induction therapy, 16 patients (67%) achieved CR/Cru, 5 PR (21%), 2 were NR (8%) and 1 was not evaluable because of toxic death after the first course. Grade III-IV anemia, thrombocytopenia and neutropenia were recorded at least after one HD-MACHOP-R course in 10 (42%), 18 (75%), and 24 patients (100%), respectively. Grade III-IV mucositis was observed in 2 patients (8%). One patient died during induction for a fatal infection. Sixteen patients (67%) performed ASCT. After transplant, 15 (94%) patients were in CR and 1 remained in PR. No transplant-related mortality was recorded. At present, after a median follow-up period of 31 months (range 2-54), 20/24 patients are alive and 19 in CR. Delayed post transplant toxic events were 1 case of Pneumocystis Carinii pneumonia and one of infectious pneumonia. No late deaths, secondary tumours or other delayed complications were observed. The projected 4 years PFS and OS of the entire patients populations (IPI HI and H) are 65.5% and 79.5%, respectively. Discussion. The results of this study compare well with historical controls and data from literature, suggesting that the administration of an intensified full induction therapy followed by consolidation with ASCT may help to improve the therapeutic outcome of patients with poor risk B-DLCL.
SAVAGE CHEMOTHERAPY IN HODGKIN'S LYMPHOMA: A SINGLE CENTER EXPERIENCE
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Introduction. Hodgkin's Lymphoma (HL) patients (pts) with relapsed/refractory disease after standard first line therapy (e.g. ABVD plus involved fields radiotherapy - IFRT) are candidates to salvage therapy with platinum- or gemcitabine-based regimens followed by autologous hematopoietic stem cell transplantation (ASCT). Retrospective analysis of DHAP and DHAP-IEGV efficacy as debulking therapy before ASCT in refractory HL pts is the object of the present report. Patients Study population comprised 23 HL pts (11 males, 12 females; median age 27 years, range: 16-47). Stage at diagnosis was II, III and IV in 5 (22%), 10 (43%) and 8 (35%) cases, respectively. 56% of pts had B symptoms, 43% bulky disease and 35% extranodal involvement. Hasencerle score was low in 26% and intermediate in 64% of pts. First-line therapy included ABVD - 6 to 8 courses - plus IFRT when indicated. DHAP regimen (2 to 3 courses) as first-line salvage therapy was administered to all pts. Disease status before salvage therapy was partial remission (PR) in 3 pts (35%), stable or progressive disease (SD or PD) in 8 pts (35%), early and late relapse in 4 (17%) and 3 (13%) pts, respectively. Pts who failed DHAP (7 pts, all PD) received IGEV (1 to 3 courses). All pts underwent ASCT with BEAM as conditioning regimen. Results. Following DHAP and G-CSF stimulation, CD34+ cell median harvest was 13.8×10^6/kg (range: 5.2-48.0). Chemotherapy to apheresis median time was 12 days (range: 10-13). DHAP resulted in complete remission (CR) in 11 pts (48%), PR in 5 (13%), SD in 2 (9%) and PD in 7 (30%). 16/23 pts (9 CR, 3 PR, 2 early relapse, 2 SD) underwent ASCT. The remaining 7 PD pts received IGEV, obtaining PR and SD in 2 and 5 cases respectively, and subsequently underwent ASCT. Overall, ASCT resulted in 14 CR (61%), 7 PR (30%) and 2 SD (9%). In the DHAP group, ASCT resulted in 12 CR (75%), 3 PR (19%) and 1 SD (6%). In the DHAP-IEGV group, ASCT resulted in 2 CR (25%), 4 PR (57%) and 1 SD (14%). Overall, after ASCT neither relapses nor progresses were observed, with a median follow-up of 28 months (range: 14-105). Discussion. These data confirm that DHAP is an effective antitumoral and mobilizing regimen in patients with relapsed/refractory HL. In poor prognosis patients with progressive disease after DHAP, IGEV allowed to contain progressive disease and enabled all patients to proceed to ASCT.

RITUXIMAB INDUCES EFFECTIVE CLEARANCE OF MINIMAL RESIDUAL DISEASE IN MOLECULAR RELAPSES OF FOLLICULAR AND MANTLE CELL LYMPHOMA
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Introduction. Molecular remission (MR) is associated with improved outcome in follicular lymphoma (FL) and in mantle cell lymphoma (MCL). If MR is lost patients are at high-risk of relapse (Gribben JG, Blood 1993, Ladetto M Exp Hematol 2003, Pott C. Blood 2006). Methods. We here retrospectively describe the molecular and clinical follow-up of eight molecular relapses (M-rel) treated with Rituximab. Four patients had FL and four MCL. Seven patients received high dose sequential chemotherapy and autologous stem cell transplantation as induction treatment (four patients with Rituximab, three without Rituximab). One FL patient was treated with conventional chemotherapy + Rituximab (CHOP-R). The molecular marker identified at diagnosis and monitored as MRD was Bcl-2/IgH in four patients, Bcl1-/IgH in two patients and IgH rearrangement in two patient. All patients achieved clinical and molecular remission (MR) at the end of treatment. M-rel was defined as PCR-positivity in two consecutive samples in the absence of clinical relapse. Results. A relapse occurred in FL at four, 42, 62, 70 months, in MCL at three, six, 39 and 52 months. They were always confirmed by direct sequencing of the clonal rearrangement. Minimal residual disease was monitored by qualitative and real-time quantitative PCR. All patients received four Rituximab courses, with two-four additional infusions if still PCR-positive. Following 4-8 Rituximab courses all patients re-entered MR. No clinical relapses have been so far recorded during the whole follow up from treatment (the median follow up was 74 months) although three patients (2 FL, one MCL) had a second molecular relapse at 90, 84 and 36 months, again sensitive to Rituximab. Real-time PCR indicated that median tumor burden at molecular relapse was 9 clonal cell x10^5 (5-150/10^5). In four cases an increase of tumor burden was seen between the first sample showing molecular relapse and that taken for confirmation. After four Rituximab courses tumor burden was reduced also in patients who re-entered molecular remission only after six or eight courses. Discussion: Our results indicate that Rituximab is active against residual FL and MCL cells and suggest that molecularly tailored Rituximab delivery might represent an attractive and cost-effective option for maintenance treatment in FL and MCL.

MULTIDIMENSIONAL GERIATRIC EVALUATION IN ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: AN EFFECTIVE TOOL TO IDENTIFY A GROUP OF PATIENTS WITH THE SAME OUTCOME AS YOUNG PATIENTS AFTER IMMUNO-CHEMOTHERAPY
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Introduction. The management of elderly patients with diffuse large B cell lymphoma (DLBCL) represents a major challenge for hematologists. A geriatric multidimensional assessment (MGA) in elderly patients with DLBCL can be safely treated have been proposed but their prospective validation is still lacking. We report on the prospective use of multidimensional geriatric assessment (MGA) in elderly pts with DLBCL. Methods. The 184 consecutive patients aged >65 with a diagnosis of DLBCL seen at our Institution from January 1995 to December 2006 were considered. The decision to treat patients intensively with a curative approach (CHOP or CHOP-like regimens with or without Rituximab) vs palliation with radiotherapy, low-dose CT or corticosteroids was based on clinical judgement throughout the entire period. From January 2003 the MGA was added to objectively assign patients to the category of frail vs fit patients. Its effect on response rate (RR), PFS and OS and its correlation with lymphoma-related prognostic factors were analysed. Results. Of 170 pts with fully evaluable data, 127 (75%) received aggressive treatment, 65/66 pts (76%) diagnosed before (group A) and 62/64 pts (74%) diagnosed after January 2003 (group B), respectively. The overall RR, 3.5 vs PFS and OS of intensively treated patients were respectively 78%, 48% and 45% in group A vs 74%, 57% and 55% in group B (p<0.5). Group B 42 pts were classified as fit (50%) and 42 as frail (50%), according to MGA. Fit pts had a significantly better RR, PFS and OS compared to frail pts : 88% vs 48% (p = 0.002), 77% vs 22% (p<0.0001) and 75% vs 20% (p<0.0001), respectively. The two groups did not differ in any pre-treatment prognostic variable, including age, IPI and stage at diagnosis. Fit pts actually received more often Rituximab than frail pts (98% vs 52% p<0.0001). Frail pts receiving intensive treatment had a similar outcome than frail pts given only palliation (2-years OS: 17% vs 25%, p= 0.9). Their toxic death rate was 5% compared to 2% with palliation (p=0.4). Group B fit pts had a borderline better survival than intensively treated pts in group A (p=0.07). Discussion. This study validates the MGA as an objective tool to prospectively select elderly pts with DLBCL, whose prognosis with aggressive immuno-CT is identical to younger pts.

PREDICTIVE VALUE OF RESPONSE ASSESSMENT WITH MID-TREATMENT EVALUATION OF 18-FDG-POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY (PET) IN AGGRESSIVE NON HODGKIN LYMPHOMA (NHL)
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Introduction. The PET is mostly used in staging of NHL patients at diagnosis and at the end of treatment. The evaluation by PET after few cycles
of chemotherapy may be useful to predict chemosensitivity and then response, progression-free survival and overall survival in this subset of patients. In our study we introduced the PET in the mid-treatment evaluation of aggressive NHL disease. Patients and Methods. From September 2005 to December 2006 25 consecutive aggressive NHL patients were evaluated for this study: 17 males and 8 females respectively with a median age of 49 years (range 21-67). We included: 18 Diffuse Large B Cell, 2 mantle cell, 1 anaplastic and 4 follicular grade III NHL. Patients characteristics were: 11/25 bulky disease; 20/25 intermediate or high IPI risk; 3/25 stage I-II and 19/25 stage IV. All pts were staged according to standard imaging procedures completed by PET at the diagnosis and at the end of treatment. After 2 or 4 cycles of immunochemotherapy PET (PET2) was repeated in all of them. Results. The PET2 findings were: 16/25 negative and 9/25 positive. The conventional and PET restaging performed at the end of treatment were 21/25 negative and 4/25 positive. Among the 16 pts PET2 negative, 14/16 remained negative at the final PET evaluation and achieved CR, 2/16 became positive with a median follow-up of 18 months: 14/16 (12%) pts PET2 negative and 1/9 (11%) pts PET2 positive respectively. Conclusions. The PET is an important imaging technique for staging and for the end-treatment evaluation of aggressive lymphoma disease, because it can better define CR pts. In the Hodgkin disease several studies demonstrated that early evaluation of the disease by PET is a crucial prognostic factor to test chemosensitivity and then to predict favourable outcome. In our study the mid-evaluation of response by this procedure had not so clear predictive value of response assessment, because patients, even if PET2 positive, can achieve CR at the end of treatment. More large studies are needed to determine the real impact of on course PET response assessment on management of aggressive NHL patients.

PO-011 MONITORING OF CAR Di TOXICITY USING BIOMARKERS IN PATIENTS WITH HODGKIN’S LYMPHOMAS TREATED WITH THE ABVD REGIMEN

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Dose-dependent anthracycline induced cardiotoxicity has been observed in long term survivors of Hodgkin Lymphomas (HL). However, early detection of patients at risk of cardiotoxicity is not possible yet. Recent data suggests that circulating biomarkers such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and Troponin I (TROP I) are sensitive and specific predictors of cardiotoxicity. Elevated secretion of NT-proBNP has been associated with both left ventricular systolic dysfunction and diastolic fillings abnormalities in patients treated with anthracycline. TROP I has also been shown to be elevated prior changes in left ventricular ejection fraction and to the appearance of cardiac symptoms. In this study we prospectively evaluated serial measurements of serum cardiac markers and echocardiography in patients with de novo Hodgkin’s Lymphomas treated with the ABVD regimen. We examined 32 patients (21 female and 11 male), median age 29,5 (range;16-64) who were scheduled to receive 6 cycles of ABVD up to a cumulative doxorubicin dose of 300 mg/m². Plasma levels of NT-proBNP and TROP I were measured at baseline and after each cycle of chemotherapy (ECHO) was performed at baseline and after the fourth and the sixth cycle of chemotherapy. In all patients baseline ECHO parameters and biomarkers were normal. During the 12 serial visits the TROP I value did not change significantly whereas the levels of NT-proBNP increased (from 83±40 to 236±100 pg/mL) in 7 patients (21,4%) during the second-third cycle of ABVD regimen after a low cumulative doxorubicin dose of 120±40 mg/m² and normalized in 5 (71.4%) of the 7 patients by the fourth cycle of therapy whereas in 2 (28,5%) patients the values remained higher than baseline up to the end of the chemotherapy. No cardiac events were documented and echocardiography did not show significant changes in the ejection fraction during chemotherapy. No significant differences in terms of age, gender and cardiotoxicity risk factors were documented between the group of patients with increased NT-ProBNP values and the patients with no NT-ProBNP increase. Our preliminary data suggest that cardiac toxicity may be detected via increased concentrations of NT-ProBNP even at low cumulative doxorubicin doses and in absence of ECHO parameters impairment. Further studies are warranted in larger populations, with a longer follow-up, to assess the significance of early NT-ProBNP increased concentrations in order to identify high risk cardio toxicity patients who may benefit from closer observation or supportive cardiac therapy.

PO-012 BONE LOCALIZATION OF HODGKIN’S LYMPHOMA: OPEN BIOPSY VERSUS FINE NEEDLE CT-GUIDED BIOPSY: A CASE REPORT

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Hodgkin disease is typically a systemic disease with involvement of cervical and mediastinal lymph nodes. In late stage HD, osseous localizations have been described in 10-20% of cases with less than 2% of cases showing skeletal lesions at the time of initial presentation. We describe a case of Hodgkin Lymphoma with initial bone involvement and persistence of the bone localisation only, even after treatment. In March 2005 a 45 year old female patient presented with pain in the left leg. Magnetic resonance imaging of the hip showed osteolytic lesions of the iliac wing and left proximal femur. These findings were interpreted as algodystrophic Paget-like alterations. In April 2005 a total body computed tomography (CT) scan showed cervical, mediastinal and abdominal lymphadenopathies, pericardial effusion, nodules in lungs, liver and spleen, the latter being also enlarged. In July 2005 a lymph node biopsy was performed and a diagnosis of Langherans cells histiocytosis was made. In December 2005 the lymph node was reviewed by a different pathologist who diagnosed nodular sclerosis, mixed cellularity variant, Hodgkin Lymphoma. Bone marrow trephine was negative. Therefore, the patient underwent polychemotherapy according to the ABVD protocol (six courses). The CT scan performed after the sixth course showed submandibular, jugular and supraclavicular lymph nodes and total regression of the hepatic, pulmonary and splenic nodules. FDG-Position Emission Tomography (FDG-PET) revealed an area of increased metabolism in the left hip. Two attempts of fine needle biopsy have been later made, but without evidence of disease. The patient therefore received two courses of DHAP. Mobilization of peripheral blood stem cells (PBSCs) failed. Subsequent FDG-PET showed persistence of increased metabolism in the left hip. She underwent polychemotherapy according to the CVD protocol. Despite the above mentioned complications and the low cumulative doxorubicin dose, this third line of chemotherapy the pain in the left hip reappeared. An open biopsy of the lesion was performed and it finally showed groups of giant anaplastic cells and Reed Sternberg cells among histiocytic cells. Lymphocytes and neutrophils were not observed. The diagnosis of an HD was confirmed by immunohistochemistry (CD30+, MUM-1, CD68 Kp1). At present the patient is receiving local (bone) radiation therapy and future therapeutic plans include high dose chemotherapy followed by reinfusion of PBSCs. To date, only a few cases of osseous HD have been reported in the literature. In the majority of these cases, similar diagnostic problems have been described. The most frequent misdiagnosis was osteomyelitis, clinical and radiologic features being quite similar. The radiologic features of osseous HD have been described as osteosclerotic, osteolytic or mixed osteosclerotic/osteolytic and may show peristomal new bone formation. In general, the radiographic findings are not path-breaking in HD. Differential diagnoses include, beside primary sarcomas of the bone, non-Hodgkin lymphomas, leukemia, metastasis, and, above all, osteomyelitis. The histologic diagnosis of osseous HD may be very problematic, undoubtedly related to the rarity of presentation of HD in bone. Nevertheless, this disease should be considered, because with the use of a more tightly focused immunohistochemistry the diagnosis is straightforward. The use of MRI, CT-scan, and FDG-PET is essential to ascertain the correct clinical stage, because the clinical stage has a critical role in the selection of treatment. The therapeutic strategies in osseous HD vary from intrasosseal surgery in the past to multimodal treatment nowadays. With the current combined polychemotherapy and radiotherapy the long term prognosis of patients with osseous HD appears better than in the past.
DIFFERENT STEM CELL DAMAGE FROM THREE CHEMOTHERAPY REGIMENS FOR ADVANCED HODGKIN’S LYMPHOMA (HL)  
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Background. To evaluate the toxicity given to the stem cell compartment by each of the three chemotherapy regimens randomly assigned to the patients with advanced stage HL enrolled into the HD 2000 GISL trial: ABVD (A), BEACOPP (B) and COPPEBVCAD (C). Materials and Methods. A cytofluorimetric measurement of bone marrow mononuclear cells stained with anti-CD34 and anti-annexin V antibody was performed 1 to 3 days before start, 40 to 50 days after stop of chemotherapy and, in a few patients, 6 to 9 months later. Per cent of marrow mononuclear cells positive to anti-CD34 (34⁺), to anti-annexin V (AV⁺) or both (34⁺AV⁺) and per cent annexin V positive of the CD34 positive cells (AV⁺/34⁺) were determined. Results. We evaluated 14 patients treated with A, 13 with B and 11 with C. Late determinations were made in 6, 8 and 7 patients, respectively. No differences were found among the basic values of the cytometric parameters in relation to the main clinical and laboratory characteristics recorded before therapy (lymphomatous marrow involvement included). When measured early after therapy, per cent of 34⁺ cells was significantly higher in the arms A and B, tending to maintain higher values in time. AV⁺ cells showed a mild increase after therapy A and B and a significant increase after C, with a trend to late lowering in each therapeutic arm. The proportion of 34⁺AV⁺ cells increased early after therapy in all the three arms, but significantly after B therapy only, tending to higher, not significant, late values, while the percentage of apoptotic elements among stem cells (AV⁺/34⁺) showed mild, not significant decrease in the A and B arm, slight increase in the C one. Conclusions. All the tested regimens tend to temporarily increase the proportion of apoptotic stem cells related to the whole amount of mononuclear cells and the one donor is a variable parameter (34⁺/34⁻ el), though the apoptotic fraction of the 34⁺ cells decreases. The C regimen increases both percentages, so demonstrating higher toxicity on bone marrow cells.

MODIFIED R-DAOX AS SALVAGE THERAPY IN PATIENTS WITH RELAPSED OR REFRAC TORY DIFFUSE LARGE B-CELL LYMPHOMA  
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Standard salvage chemotherapy for aggressive non Hodgkin’s lymphoma has not been established. DHAP has been one of the most effective and used regimens. This study was designed to assess the efficacy and safety of substituting cisplatin with oxaliplatin in the DHAP regimen for patients with relapsed or refractory high grade non Hodgkin’s lymphoma on outpatients basis. Patients (pts) relapsed after first line or salvage therapy (ABMT) or primary refractory were treated at three weekly intervals with Rituximab (375 mg/m² day 0), oxaliplatin (120 mg/m² day 1), cytarabine (2000 mg/m² days 2,3) and dexamethasone (40 mg days 1 to 4). Fourteen pts were programmed to receive peripheral blood stem cells transplantation and 23 did not. Thirty-seven pts with median age of 59 years (range 33-75) entered this study. Histological subtypes were 34 diffuse large B cell and 3 grade III follicular lymphoma. The overall response rate (RR) was 78% (29/37) including 15 complete remission (CR) and 14 partial remission (PR). Thirty-one pts were treated with R-DAOx as second line, six pts were treated with R-DAOx as third line therapy (4 were relapsed after ASCT) and five obtain a response to therapy. Ten out fourteen (71%) pts programmed to high dose therapy obtained a response and were treated with PBSTC. Eleven pts were primarily refractory and only four obtained a partial response with R-DAOx. In a univariate analysis chemosensitive disease and PS 0-1 at salvage therapy were significantly correlated with response to therapy. The majority of pts experienced severe haematological toxicity despite the use of hematopoietic growth factors, none of them required hospitalisation. No grade 3-4 extra-hematological toxicity was reported, in particular we did not observe any significant renal and neurological toxicity. During a median overall survival (OS) period of 12 months (range 2-39 months) 11 pts died (30%). Probabilities of 1-year progression free survival (PFS) and were 34% and 48% respectively. If we consider only chemosensitive pts, after R-DAOx, the PFS and OS were 48% and 63% respectively. The only one factor significantly correlated with better OS was response to therapy. R-DAOx is a novel combination for the treatment on outpatients basis relapsed or refractory pts with aggressive lymphomas. It has a clinically significant activity in chemosensitive pts with an acceptable toxicity profile that makes this regimen attractive before high-dose chemotherapy.

LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF LYMPHOMA PATIENTS  
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Myocet (liposomal doxorubicin) has an improved pharmacokinetic profile with less myelosuppression and GI toxicity and has a reduced risk of cardiotoxicity at dose level equivalent to standard formulations of doxorubicin. From June 2003 we replaced the conventional doxorubicin with liposomal doxorubicin (Myocet 50 mg/m² in COMP and 25 mg/m² in MBVD) for the treatment of 56 patients (pts). They were selected: elderly pts, pts with impaired cardiac function, pts previously treated with doxorubicin. Thirty-one pts with NHL were treated with R-COMP and 5 Hodgkin’s lymphoma with MBVD. The median age was 68 years (range 54-76). Fourteen pts were stage I-II and 22 were stage III-IV. According to histology: 27 were DLBL, 3 mantle cell lymphoma, 1 PTCL. According to IPI score, for NHL only, 25pts (81%) presented IPI2. Eight were pretreated with doxorubicin (580 mg median cumulative dose), 20 pts showed impaired cardiac function (2 ischemic, 9 hypertensive and 3 hypokinetic). The median left ventricular ejection fraction (LVEF) at diagnosis was 58% (range 35-70%). We performed cardiac evaluation at diagnosis, after three cycles and at the end of therapy. All pts but one had no change in LVEF, one patient (3%) presented a myocardial disfunction resolved with medical therapy. The average dose of liposomal doxorubicin for patients who concluded therapy was 465 mg (range 30-600 mg). At the moment 34 out 36 pts are evaluable for response: 27 pts obtained a complete remission (79%) five a partial remission with an overall response of 94%. Five out eight (62%) pretreated pts reached a new complete remission. After 180 cycles according to toxicity one patient stopped therapy due to myocardial disfunction and two patients died one for a stroke and the other for gastrointestinal bleeding. No significant hematological toxicity was recorded. Three pts died of disease and after a median observation period of 12 months (range 1-32) the overall survival was 80%. We conclude that liposomal doxorubicin is effective in very pretreated patients with concomitant diseases which could limit the use of conventional anthracyclines. Myocet is feasible and effective in a subset of patients with very negative characteristics at diagnosis. It reduces cardiotoxicity risk without reducing chemotherapy efficacy. Also in pts heavily pretreated R-COMP regimen was effective with an overall response rate of 100%.

ANTHRYCYCLINES BASED CHEMOTHERAPY PLUS SEQUENTIAL RITUXIMAB AS FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA: LONG TERM FOLLOW-UP RESULTS  
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The use of anthracyclines in the treatment of follicular lymphoma is controversial. The advent of monoclonal anti-CD20 antibody has increased the possibility for the treatment of indolent follicular lymphomas. The lack of myelotoxicity indicates that Rituximab would be well suited either as a single agent or in combination with chemothera- py. Starting from 1999 until december 2003 we have used Rituximab after induction therapy with anthracyclines based regimens in 51 patients with diagnosis of follicular lymphoma to enhance the ADCC effect. The characteristics of patients were the following: 16 female and
35 male; median age 52 years (range 30-76); 18 patients were in stage I-II, 33 stage III-IV; according to FLII 23 were low-risk, 15 intermediate-low risk, 13 intermediate-high risk and 1 high risk. Fourteen patients (27%) had histological positive bone marrow; bulky disease was present in 8 patients; pathologic LDH value in 12 patients. All patients were treated with antracyclin containing regimens (CHOP or CHOP-like) for six cycles and than with Rituximab at the dosage of 375 mg/m² weekly for four weeks. Forty-eight patients (94%) obtained a complete remission (CR) and 3 a partial remission with an overall response rate of 100%. No severe hematological or extrahematological toxicity were observed either with chemotherapy or with immunotherapy. Two patients was died because of disease progression and after a median follow-up of 65 months (range 18-97) the overall survival was 96%. Fifteen patients (31%) experienced a relapse and after a median period of 46 months (range 7-89 months) the disease-free survival (DFS) was 69% with 26 patients at risk. The mean period for relapse was 25 months (range 7-45 months). No characteristics at diagnosis analysed was significantly correlated with better DFS. Seven out 15 relapsed patients obtained a new complete remission and 5 remained persistently in second complete remission. This study confirm the safety, feasibility and efficacy of this procedure. The long follow-up confirms that Rituximab in a sequential program could increase the relapse free survival probably reducing the minimal residual disease. Obviously this data should be confirmed with long-term results of Rituximab maintenance therapy. The use of maintenance will reduce the rate of relapses that also in our study is clinically relevant.

PO-017
INTRATHECAL LIPOSOMAL CYTARABINE AS THERAPY OF LYMPHOMATOUS MENINGITIS
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Introduction. The Lymphomatous Meningitis (LM) represents an event with a generally poor prognosis and a median survival of few months in untreated patients. Its occurrence is tightly related to the histological type, the response to the therapy and the introduction of a proper prophylaxis in the treatment plan. Recently, an important role both in the prophylaxis and in the therapy of LM is played by the intrathecal administration of Cytarabine in liposomal formulation (DepoCyte): randomized studies have showed a significant better effectiveness and a reduced toxicity of liposomal formulation treatment with respect to the traditional one. Methods. In this work 14 cases of LM treated with systemic chemotherapy and DepoCyte (50 mg IT every 15 days x 6 times) are shown. Male/Female ratio is 9/4; average age is 43 years (range 24-78). In all patients neurological symptoms were present; lymphomatous cells were identified in the liquor of 7 patients and Central Nervous System (CNS) localizations were detected by NMR in 9 patients. Results. The 7 CSF-positive cases were heterogeneous, as follows: 1) B-lineage CD10+ ALL meningeal relapse; 2) Lymphoblastic T Lymphoma meningeal localization, with mediastinic mass; 3) DLBCL meningeal localization; 4) LM at diagnosis in a 78 years old patient; 5) Acute Myeloid Leukemia M3 FAB with meningeal relapse; 6) Mantle Cell Lymphoma meningeal (and systemic) relapse; 7) Multiple Myeloma with meningeal localizations. In the first 5 patients CR was obtained and they are still alive; only the patients no. 6 died for disease progression. The patient no. 7 is still in treatment. The remaining 7 CSF-negative cases had been diagnosed as DLBCL (4 at diagnosis and 3 at relapse) CNS localizations. 5 patients had a good response to treatment and 3 are still alive and in CR. One patient is still in treatment. Discussion. Overall, DepoCyte treatment was shown to be mostly effective, well tolerated by all patients and devoid of undesired side effects. The association with various systemic chemotherapy protocols had been demonstrated to be suitable and endowed with synergistic effects. Studies with higher number of patient might validate the effectiveness of DepoCyte also in those CSF-negative cases with solitary CNS localization.

PO-018
FINAL REPORT 5OF GISL LL02 TRIALS FOR THE TREATMENT OF LOW-GRADE NON-FOLLICULAR NON-HODGKIN LYMPHOMA, AT DIAGNOSIS
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Indolent Non-follicular non-Hodgkin Lymphoma (Nf-NHL) is a group of relatively frequent lymphoid neoplasms, characterized by diseases, nevertheless extended clinical and prognostic studies are still lacking. In 2002 the Gruppo Italiano Studio Linfomi (GISL) initiated a LL02 prospective multicenter phase II trial, with the aim to evaluate the efficacy and safety of FC combination in the first-line therapy of Nf-NHL patients younger than 70 years. Between July 2002 and September 2006, 58 adult patients (35 males and 23 females, median age 64 yrs, range 40-75) affected by Nf-NHL, at active disease phase, were consecutively enrolled in 12 GILS Hematological Centres. Patients were treated with a dose of 25 mg/m² Fludarabine plus 250 mg/m² Cyclophosphamide administered intravenously daily for 3 days; each cycle was repeated every 28 days for 6 courses. During the treatment patients received oral thrimethopri-sulphamethoxazole prophylaxis. After the intermediate evaluation, 40/58 patients (62.8%) had an objective response (ORR) with a 20.7% of complete remission (CR) plus 62.1% of partial remission (PR); at the final evaluation the ORR percentage was 64.5% with a 41.4% of CR (24 pts) and 43.1% of PR (25 pts); three patients were in progressive disease (5.2%) and one in stable disease (1.7%). The median overall survival (OS) was not reached with an 88% and 84% at 12 and 24 months; the progression free survival (PFS) was 89% and 77% and the event free survival (EFS) was 51% and 66% at 12 and 24 months respectively. About the toxicity profile, the major toxicity was hematological with a 18% of grade III or IV anemia, 40% leucopenia, 33% neutropenia and 10% piatistopenia. The 12% of patients had an infective episode with a 7.7% of WHO grade III-IV. In conclusion the FC chemotherapy is a useful chance for advanced untreated non follicular low-grade NHL, with an optimal ORR, CR and DFS. The crucial point of FC remains OS, that not seems to be significantly improved in comparison with fludarabine alone or with standard therapy, even though the better quality of responses, Rituximab plus FC association is growing in literature as the probably key to find a real improvement also in this aspect.

PO-019
AUTOIMMUNE COMPLICATIONS IN 90 PATIENTS WITH SPLENIC AND NODAL MARGINAL ZONE LYMPHOMAS
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Introduction. Splenic and nodal marginal zone lymphomas (SMZL and NMZL respectively) are rare indolent lymphomas: autoimmune complications are reported in 10 to 15% of the cases. We retrospectively analyzed our patients with SMZL and NMZL in order to assess the incidence and characteristics of autoimmune phenomena, their relationship with clinical and biological characteristics and their impact on survival. Patients and Methods. Ninety patients with non-MALT MZL, diagnosed between 1988 and 2007 at our Institution were evaluated. SMZL were 81 and NMZL 9, males 58 and females 32 (M/F 1.81), aged 34 to 86 years (median 63.5). In 84% of SMZL patients splenomegaly was present, all the NMZL patients had peripheral lymphadenopathy. Anti-HCV seropositivity (RIBA) was detected in 14/70 (20%) tested patients. Results. Clinically relevant autoimmune complications occurred in 21/90 patients (23.3%): 8 cases of autoimmune haemolytic anaemia (AIHA),
3% of autoimmune thrombocytopenia, 2 of Evans’ syndrome, 3 of anti-MA2-associated nephropathy, and 1 of rheumatoid arthritis, Sjogren’s S. and symptomatic cryoglobulinemia respectively. One patient presented both AIHA and antiphospholipid syndrome (APS) and another autoimmune thrombocytopenia and APS. We also found 7 cases of clinically asymptomatic cryoglobulinemia and 9 patients with Rheumatoid factor or ANA positivity. Incidence of autoimmune complications was higher in females (p<0.05); no significant correlation was found between autoimmune disorders and: age, presence of splenomegaly, LDH or beta2-microglobulin levels, anti-HCV seropositivity and leukemiac presentation. In 14/21 patients autoimmune complications occurred at presentation or within 3 months from diagnosis; in 4 they preceded the diagnosis of lymphoma of 94, 60, 18 and 3 months respectively. Treatment of symptomatic autoimmune complications consisted of: steroids (14 patients) associated with alkylating agents (7) or splenectomy (5), Rituximab + polichemotherapy (5), other drugs (2). Median overall survival of all patients was 106 months; the occurrence of autoimmune complications did not significantly influence survival. Conclusions. In our series of SMZL and NMZL the incidence of autoimmune phenomena was high.

**PO-020**

**ANTIVIRAL TREATMENT WITH INTERFERON ± RIBAVIRIN AFTER CHEMOTHERAPY FOR DIFFUSE LARGE B-CELL NON-HODGKIN LYMPHOMAS WITH HEPATITIS C VIRUS (HCV) INFECTION**


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We have previously shown that diffuse, large B-cell lymphomas (DLBCL) are HCV+ in about 12% of cases in Italian population. These patients show peculiar clinical characteristics and have an outcome generally not significantly different, in terms of response rate, progression free survival (PFS) and overall survival (OS), from that of subjects with HCV+ DLBCL, when treated with standard or even high dose chemotheray (CT) and if significant signs of liver dysfunction are absent. Antiviral therapy (AVT) with interferon ± ribavirin has shown to be effective in inducing neoplastic regression without CT in low-grade HCV+ non-Hodgkin’s lymphomas. In the present study we aimed to determine the possible role of AVT, performed after a standard CT treatment, in HCV+ DLBCL. We evaluated the clinical outcome of 33 HCV+ DLBCL patients who received AVT (alpha or pegylated interferon ± ribavirin, at recommended doses and therapy duration for specific HCV genotypes and according to viral response) after first complete or partial remission was achieved by front-line standard CT. CHOP/CHOP-like ± rituximab or PRMACE-Cytarabine were generally employed. For comparison, a historical cohort of 29 HCV+ DBLCL, receiving similar CT, but without AVT, was employed. The two groups were similar for age, sex, clinical stage, liver function, type of prior CT, viral load and HCV genotype. Sequential treatment (CT followed by AVT) was generally well tolerated. Four patients, however, interrupted AVT before three months due to general malaise or myelotoxicity. HCV clearance was obtained in 57% of patients. An interim evaluation showed a not statistically significant cure rate (60% vs 55%) in favour of AVT-treated patients in terms of PFS at three years. The same was observed for three-year OS (69% vs 58%, p.n.s.). A weak correlation between viral clearance and longer PFS and OS duration was also observed. Our currently available data indicate that a sequential treatment with CT followed by AVT is feasible in HCV+ DLBCL, may induce complete virus clearance and could have a positive impact on clinical outcome. A larger number of patients and a longer follow-up are required to establish the exact role (if any) of AVT in HCV+ DLBCL patients.

**PO-021**

**INDUCTION OF COMPLETE MOLECULAR RESPONSE WITH YTTRIUM-90 IBRITUMOMAB TUXETAN (Zevalin®) IN B CELL NON-HODGKIN’S LYMPHOMA PATIENTS AFTER THE ACHIEVEMENT OF A COMPLETE CLINICAL RESPONSE WITH CHEMOTHERAPY REGIMEN**


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Introduction. Radioimmunotherapy (RIT) is a new treatment for B non-Hodgkin’s lymphoma (NHL) patients. **Y** ibritumomab tiuxetan (Zevalin®) consists of a murine monoclonal antibody to CD20, conjugated to a metal chelator tiuxetan for retention of the beta emitter **Y**. Thus RIT is a targeted radiation therapy. NHL, combining targeting attributes of a monoclonal antibody and the beta radiation of **Y**. Zevalin is approved for the treatment of follicular lymphoma (FL) refractory to or relapsed after rituximab, on the bases of clinical trials where it achieved a response rate as high as 83%. Several ongoing registrational trials are evaluating the efficacy of Zevalin® in other NHL, as diffuse large B cell (DLCL) and mantle cell lymphoma (MCL). We are here evaluating the effect of Zevalin® as consolidation therapy in NHL patients that achieved a complete clinical response (CCR) with chemotherapy. Methods. In B cell NHL patients that achieved a CCR after 1st or 2nd line anthracyclines based chemotherapy ± Rituximab, minimal residual disease was evaluated by PCR, for the following rearrangements: JH, Bcl-1, Bcl-2. Patients received Zevalin® 6-9 weeks post chemotherapy. Evaluation of molecular response was assessed after a follow up period at 12 weeks. The aim of the study was to assess the role of Zevalin® in induction of a complete molecular response after a complete clinical response. Results. 18 B-NHL patients (10 FL, 6 MCL, 2 DLCL, male-female 9:9, median age 63, range 53-72) in a CCR after chemotherapy have been enrolled. 10 patients had a pathological rearrangement before RIT, while 8 were already in a CCR condition. Zevalin® was completed in all 18 patients and the post infusion evaluation was performed after 12 weeks. In the follow-up period grade 3 thrombocytopenia was commonly documented, but it was not associated to bleeding or need of platelet transfusion. After 12 weeks from RIT a new molecular evaluation was performed on bone marrow samples. Up to now, only 10 patients have completed the 12 weeks follow-up: all 6 patients positive before RIT achieved a CCR with Zevalin® administration. The 4 PCR negative patients maintained the CCR. Conclusions. Zevalin® is an efficient consolidation therapy in B cell NHL patients after chemotherapy. In 10 valuable patients Zevalin® administration allowed to convert 6 CCR to CMR. In the remaining 4 patients Zevalin® prolonged the CCR. Zevalin® addition to medication treatment is feasible and associated with manageable hemato logical toxicity.

**PO-022**

**18F FDG PET-CT BASED STAGING OF PRIMARY EXTRANODAL LYMPHOMAS COMPARED TO CONVENTIONAL MODALITIES**

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Aim. The aim of our study was to assess the value of FDG-PET/CT compared to conventional modalities (US, contrast enhanced CT, bone radiography, MRI and endoscopy, depending on the primary localization) in staging Primary Extranodal Lymphomas (PEL). Materials and Methods. Starting November 2005, we examined 27 consecutive patients with PEL, 19 males and 8 females, median age 53 years (range 17-76 years). All the patients had received diagnosis of Non Hodgkin Lymphoma from biopsy at the affected site. The sites affected were the following: 11 Stomach, 3 Bone, 3 Bronchus, 2 Orbit, 2 Skin, 2 Liver and 1 each Lacrimal Gland, Parotid Gland, Esophagus and Spleen; 15 patients were affected from low-grade NHL and 12 from an aggressive type. PET/CT was performed in all patients at diagnosis, and the findings were compared with those obtained from Conventional Modalities (CM). PET/CT was also performed during the follow up in 20/27 patients; also these findings were compared with CM carried out at the same time. Maximum standardized uptake values (SUV) on tumor bulk were correlated with the histotype, and with the response to treatment in the follow up scans; a SUV reduction ≥25% was considered a partial

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response. Results. At diagnosis, both PET/CT and CM showed concordant findings in 22/27 (85%); in the remaining 4 patients, all with positive CM (1 gastric, 1 bone, 1 bronchus, 1 gastric) with significant reduction of SUV max at PET/CT scans. Up to March 2007, all 20 patients were in clinical remission (11 lymphoma, 9 haematological). In 20/27 (74%) no tracer uptake was observed, and CM findings were concordantly negative. 5 patients (25%) resulted positive at CM (2 bone, 1 bronchus, 3 gastric) with significant reduction (56±26%) of tracer uptake at PET/CT scans. 4 patients (20%) resulted positive at CM (2 liver, 1 esophagus, 1 bone), while PET/CT scan was negative. Conclusions. At staging, PET/CT showed good sensitivity (85%), thus representing an accurate method for lymphoma characterization also in the subset of patients with primary extranodal lymphoma. The SUV evaluation may be a useful tool in the course of the disease for the evaluation of the response to treatment; uptake disappearance or a marked reduction of SUV max seems identifies correctly the patients in clinical remission.

**PO-024**

**SURFACE ANTIGEN MOSAIC OF DENDRITIC LYMPHOPLASMACYTOID LYMPHOMA: A KEY FOR INTERPRETING THE HETEROGENEOUS TISSUE LOCALIZATION OF NEOPLASTIC CELLS**

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During the last years an unusual form of lymphoma whose cells expressed HLA-DR, CD4, CD56 and IL2R was described. Functional and phenotypic properties of these cells were similar to a fraction of normal dendritic cells, the DC2 or lymphoplasmacytoid subset. The clinical behavior of this disease generally implies skin involvement with an aggressive outcome and possible picture of acute leukemia. Two main types of patients have been described: (i) cases with exclusive bone marrow involvement and (ii) cases with initial skin involvement and subsequent leukemic dissemination. In the study we characterized 10 cases of dendritic lymphoplasmacytoid lymphoma (DLPL) by using an extended panel of MAbs, flow cytometry (FCM) and immunohistochemistry (IH). Six cases were studied by FCM and 5 by IH. The aims were: (i) to define the exact immunophenotype of this rare neoplasia; (ii) to assess the real incidence of these cases; (iii) to attempt an immunophenotypic sub-classification. FCM characterization, performed on bone marrow aspirates in 5 cases with heavy bone marrow involvement, consistently evidenced the phenotype HLA-DR+, CD4+, CD56+, CD103+. One case with massive involvement of bone marrow and no cutaneous infiltration clearly showed CD26. All cases were initially referred to our institution as acute myeloblastic leukemia (AML). The exact incidence of these cases as compared to all cases of AML studied was 5 out of 2158. IH was performed on cutaneous biopsies in 5 patients, all of which expressed CD4, CD56, CD123 and TCI1, along with variable expression of CD68 and CD43. In one case more than 50% of cells expressed TRAIL. FCM also showed CXCR4 (CD120a). This receptor and its ligand CXCL12/SDF1 seem to drive metastasis in several cases of neoplastic disease. CD26/CDPIV seems to counterbalance CXCR4 function based upon its enzymatic activity on CXCL12. In this study, CXCR4 was observed by IH in all cases, while convincing expression of CD26 was never found. One cutaneous fragment was studied by FCM, confirming the CXCR4+CD26+ pattern. In conclusion, immunophenotype of this rare disease seems to be stable as regards HLA-DR, CD4, CD56, CD56 and CD103 display. CXCR4 and CD26 appear to be associated with tissue distribution of neoplastic cells, with CXCR4+CD26- pattern corresponding to cases characterized by initial cutaneous involvement and metastatic potential, CD26 bright expression being restricted to bone marrow disease.

**PO-025**

**EFFICACY OF RADIOMUNOTHERAPY (RIT) WITH \textit{\textgreek{y}-IBRITUMOMAB TIUXETAN (ZEVALIN) FOR THE TREATMENT OF RELAPSED OR RESISTANT AGGRESSIVE LYMPHOMA HEAVILY PRETREATED WITH RITUXIMAB + CHEMOTHERAPY**

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Chiappella A,
Fioritoni G,
Freilone R,
Martelli M,
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Introduction. The addition of antibody monoclonal anti CD20 Rituximab to CHOP chemotherapy has resulted in improved CR, progression free survival and overall survival rates for patients affected by follicular
lymphoma. Recently, a phase II trial has showed that Zevalin has a useful activity in the treatment of relapsed refractory elderly DLBCL. We report the results of a study to evaluate the clinical utility and safety of Zevalin in relapsed or chemoresistant aggressive lymphoma. **Patients and Methods.** Patient eligibility was represented by: age over 18 yrs, refractory or chemoresistant aggressive lymphoma (grade III follicular, PML or DLBCL de novo), CD20 positivity, WHO performance status of 0 to 2, stage II bulky, III or IV, bone marrow involvement < 25%. All patients were previously treated with almost two lines of chemotherapy and all of them received Rituximab. All patients signed a written informed consent approved in accordance with institutional guidelines. Patients with partial or complete response (>50% and >90% respectively) to chemotherapy + ASCT as salvage therapy. Larger studies are needed to confirm this preliminary data. **Discussion:** Rituximab was safe and effective in combination with chemotherapy in the treatment of chemorefractory DLBCL. These findings prove that chemoimmunotherapy with Rituximab + chemotherapy can be considered an effective therapeutic approach for patients with relapsed diffuse large B cell lymphoma.
allogeneic stem cell transplantation. Median age was 48 years (range 21-73); all FAB subtypes were represented with the exception of M3 cases. We studied 58 and 56 pairs of BM and PB at the end of induction and consolidation therapy, respectively. Results. Findings in BM e PB were significantly concordant after induction and consolidation therapy. Median value of BM residual leukemic cells (BMRLC) and PB residual leukemic cells (PBRLC) after induction, were 5.2×10⁴ (range 1×10⁹-2.2×10⁴) and 2.85×10⁴ (range 1×10⁴-1.15×10⁴), respectively (p=0.84, p<0.001). After consolidation, the median value of BMRLC and PBRLC were 4.2×10⁴ (range 2×10⁴-7.27×10⁴) and 3.85×10⁴ (range 1×10⁴-1.34×10⁴), respectively (p=0.85, p<0.001). Using the maximally selected log rank statistics, we found that the cut-off of 1.5×10⁴ PBRLC correlated with the disease outcome. In fact, 36 of 50 (72%) patients with PBRLC > 1.5×10⁴ after induction had a relapse, whereas, the 8 patients with <1.5×10⁴ did not (p<0.001). After consolidation, 45 patients had a level of MRD > 1.5×10⁴ and 54 (76%) had a relapse; 10 out of the remaining 11 patients whose level of MRD was below 1.5×10⁴, are still relapse free (p<0.001). The median duration of relapse free survival (RFS) was not reached among the patients with a PB MRD negative status after consolidation, whereas it was 9 months among those who tested PB MRD positive after consolidation (p=0.001); the multivariate analysis confirmed the independent prognostic role of the PB MRD status at the end of consolidation (p=0.019). Discussion: 1) MRD is detectable and measurable in PB of AML patients using MPFC; 2) the levels of MRD in BM are correlated to those measured in PB; therefore PB may be a complementary source for MRD studies in patients with AML; 3) the PB MRD determination after consolidation therapy has a prognostic role; 4) the combined BM and PB assessment might optimize MRD monitoring in AML, thus allowing risk-category stratification to be improved.

PO-028 CLEANSING OF BLASTS FROM PERIPHERAL BLOOD DURING INDUCTION TREATMENT PREDICTS THE BONE MARROW RESPONSE IN ACUTE MYELOID LEUKAEMIA Gianfaldoni G,1 Manelli F,1 Baccini M,4 Antonioli E,1 Bencini S,1 Leoni F,1 Bosi A1
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Introduction Although several parameters (i.e. age, cytogenetics and secondariness) are useful for risk stratification of patients with acute myeloid leukaemia (AML), there are no firm criteria for predicting response to induction treatment of individual patients. Aims To predict the individual response in a clinically relevant time, we analysed the clearance of peripheral blasts (PBC) in 57 AML patients during 3+7 induction course. Methods By extensive flow cytometry (FC), a population of cells with leukaemia-associated aberrant immuno-phenotype (LAIP) was identified in each patient from the initial bone marrow (BM) aspirate. We then obtained LAIP-positive absolute blast counts on peripheral blood (PB) immediately before starting therapy (day 1) and every day until day 5. PBC was expressed as the ratio, converted to logarithmic scale, between baseline value (day 1) and daily absolute blasts count. At day 14, FC analysis was performed on BM in order to identify LAIP-positive residual blasts. The degree of BM clearance was expressed as the ratio, converted to logarithmic scale, between the percentage of LAIP-positive blasts determined at diagnosis and day 14 (LD14). Results Between May 2004 and January 2007, 57 consecutive newly diagnosed non-M3 AML patients aged less than 66 years entered the study and were evaluable for BM response. After a single course, complete remission (CR) was achieved in 31 patients. CR was not obtained in 27 patients (NCR), 17 of whom were refractory.

Table 1

<table>
<thead>
<tr>
<th>Day</th>
<th>NCR Median (95% CI)</th>
<th>CR Median (95% CI)</th>
<th>Rank Sum</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.16 (10.5, 0.38)</td>
<td>0.46 (0.38, 0.57)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.52 (50.1, 1.0)</td>
<td>1.19 (11.1, 1.54)</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.11 (0.91, 1.67)</td>
<td>2.25 (2.05, 2.56)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.60 (1.34, 2.2)</td>
<td>2.80 (2.71, 3.41)</td>
<td>&lt;.0001</td>
<td></td>
</tr>
</tbody>
</table>

According to conventional criteria (cytogenetics and secondariness) there were 17 high risk patients, of whom 4 achieved CR, 35 interme- 
diate risk patients, of whom 22 achieved CR, 5 low risk patients, all of whom achieved CR. The ranges of distribution of PBC had minimal overlap between CR and NCR groups. The median reduction in the two groups were significantly different on each day (Table 1). The rate of PBC appeared higher in CR than NCR patients with an estimated difference between groups equal to 0.29 (95% CI: 0.14-0.44; p-value<0.001). This difference was not attributable to differences in baseline PB leukemia burden and assigned risk. PBC showed an excellent correlation with BM response as assessed by morphologic analysis at haematopoietic recovery and by FC on day 14. Specifically CR was achieved only in 1 of 19 patients (5%) who had a PB below 2 logs on day 5, whereas CR took place in 29 out of 38 patients (76%) who had a PBC greater than 2 logs on day 5. Higher values of PBC on each day were associated with larger LD14. This correlation was significant on each day and it increased monotonically over days. Summary/conclusions These data indicate that PB may be in equilibrium with BM in each AML patient, and that PB clearance gives evidence of BM clearance. Therefore, a major treatment outcome may be predicted very early during the induction therapy of AML patients, thus providing an opportunity to tailor treatment modalities since the outset.

PO-029 TRIAZENE COMPOUNDS AND MGMT INHIBITORS IN ACUTE LEUKEMIA: PRECLINICAL AND CLINICAL STUDIES Marchesi E,1 Turriziani M,1 Venditti A,1 Caporaso P,2 Tortorelli G,1 Buccisano F,2 D’Atti S,2 Tirindelli MC,2 Amadori S,1 Bonmassar E1
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Previous preclinical and clinical studies indicated that triazene compounds (i.e. dacarbazine and temozolomide, TMZ) could be highly effective against refractory acute leukemia. Antitumor activity of these compounds is mainly based on the generation of methyl adducts to purine and pyrimidine DNA. The DNA repair protein, O6-methylguanine-DNA methyltransferase (MGMT), and the mismatch repair (MMR) system, play a pivotal role in the cytotoxic effects of triazenes. In particular, high levels of MGMT are responsible of target cell resistance to these compounds. On the contrary, the presence of MMR is required for the cytotoxic effects of triazenes. Preclinical studies performed in our laboratory demonstrated that in vitro suppressive effects of TMZ against human leukemia blasts could be potentiated by pre-treatment of target cells with MGMT inhibitors, such as O6-(4-bromomethyl)guanine (lomeguatrib). In the early seventies we discovered that, in vivo, triazene compounds induced the appearance of novel transplantation antigens in murine leukemia (chemical xenogenization, CX). Non-self peptides presented by class I MHC molecules are generated by triazene-induced somatic mutations, affecting retroviral sequences that are detectable in the mouse genome. Moreover, preliminary experiments suggested that human cancer cells can also undergo CX. Therefore, a pilot study for patients with relapsed/refractory acute leukemia is now in progress. So far, we have treated 8 patients (6 with AML and 2 with ALL) with TMZ (150 mg/m²/day orally for 7 days) + lomegutrib (that suppressed MGMT activity) to obtain a cytoreduction of sensitive leukemia blasts and to presumably induce CX in resistant blasts. After chemotherapy we treated these patients with IL-2 immunotherapy (0.5 MU/day s.c. for 1 month) to possibly restore the immunogenicity of the remaining disease (resistant blasts) of the hypothetical triazene-induced CX. The results show that lomeguatrib suppressed MGMT activity in vivo and in vivo in all patients entered into this protocol. In fact, 6 of 8 patients experienced complete (5 cases) or partial (1 case) disappearance of leukemia blasts in peripheral blood and in bone marrow. Whereas non-hematological toxicity was limited, we observed a severe and long-lasting myelosuppression. MMR proteins were found to be expressed in blasts of 4 out 4 patients tested (1 of these patients is resistant to treatment and in this case a functional alteration of MMR can be hypothesized). At the present time, 2 patients survived more than 9 months and 1 is still alive, 4 patients died because of opportunistic infections and 3 for progressive disease. This investigation confirm the potential role of triazenes in acute leukemia and highlights the role of drug-induced MGMT suppression in overcoming resistance to these agents. Further studies are necessary to establish different
approaches to control minimal residual disease and to clarify a possible clinical applications of triazenes in patients affected by others hematological malignancies refractory to conventional treatments, such as multiple myeloma and plasma cell leukemia.

**PO-030**
**EVALUATION OF TELOMERASE ACTIVITY IN PATIENTS WITH ACUTE LEUKEMIAS**
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Background. Human telomeres are specialized chromosomal and structures composed of TTAGGG repeats. They protect chromosomes from degradation by fusion and recombination. However, in normal cells, the number of cell division is limited and the main reason for that is the progressive loss of telomeric sequences. This process leads also to cell senescence when a critical telomere length is reached. The complete replication of telomeric sequences at the termini of eukaryotic chromosomes requires a special enzyme complex, telomerase, an RNA-dependent DNA polymerase, which is not present in most somatic cells (only immortalized cell lines and activated lymphocytes have borderline telomerase activity). In contrast, immortalized cell lines and the majority of malignant tumors demonstrate high telomerase activity, stable telomere length and unlimited proliferative potential.1 We analysed the telomerase activity in 35 AML and 8 ALL patients. Moreover 30 normal subjects were evaluated. Methods. Fluorescence TRAP assay was performed on bone marrow samples as previously described2 and expressed in arbitrary enzymatic units (AEU). Results. 35 pts with AML (19 M and 16 F) were evaluated at diagnosis. Their median telomerase value was 185 AEU (extr. 48-2723). 16 pts were evaluated after therapy. Their median post-therapy value was 61.5 AEU (extr. 28-244). The 8 pts with ALL had a median telomerase value at diagnosis of 532 AEU (extr. 35-690). After therapy, the median value was 52.5 AEU (extr. 35-61). Normal controls showed a median value of 94.0 AEU (extr. 46-346). No clear-cut correlation was observed with age, WBC count or karyotype. A dramatic response with reduction to normal values was observed in all cases studied both before and after therapy, even in those who did not achieve a complete remission. Conclusions. These data confirm previous data of the literature: increase in telomerase activity both in AML and ALL, with a reduction within normal range after therapy.

Acknowledgements. This study was carried out with the support of the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola-Forlì and of the Ravenna section of the Associazione Italiana contro le Leucemie, Linfomi e Mieloma (Ravenna A.I.L.).

References

**PO-031**
**ONE MUTATION MODEL CAN EXPLAIN AGE INCIDENCE IN ACUTE LEUKEMIA WITH MUTANT NUCLEOPHOSMIN GENE**
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Background. Analysis of age-specific incidence can uncover the number of genetic events needed to cause neoplasia, but the dynamics of cancer initiation and its epidemiological consequence can vary in different tumours. It is widely accepted that in acute myeloid leukemia (AML) a single genetic event (e.g. chromosomal translocations) is not sufficient to cause the disease. However, AML is molecularly and clinically a heterogeneous disease and recently a large and homogeneous subgroup was identified: AML patients carrying mutations of the nucleophosmin (NPM) gene (NPMc+ AML). Whether NPM mutation is necessary and/or sufficient to cause leukaemia is unknown. Methods. We collect data sets at four major European Institutions (Laboratory for Molecular Diagnostics, University Hospital Carl Gustav Carus, Dresden, Germany, MLL’- Munich Leukemia Laboratory, Munich, Germany; Department of Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands; Institute of Hematology, University of Perugia, Italy). We design a mathematical model in which we consider a population of N wild-type cells with relative fitness r = 1 following the Moran Process. Results. First we evaluate the dependence of age-specific NPMc+ AML rates on country. Linear regression of the curves shows similar slopes. In particular on a double-log scale, all data have a slope of about 4. Then we demonstrate that a one-mutation mathematical model can explain this result (Figure 1). Conclusions. The exponential phenotype of NPMc+ AML can be explained by a single mutation.
**PO-032**

**BIOLOGIC AND CLINICAL SIGNIFICANCE OF CD34 EXPRESSION IN ACUTE PROMYELOCYTIC LEUKEMIA**


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Patients with APL disclose a typical immunophenotypic pattern in their leukemic cells and a sizable proportion of cases are positive for the early hematopoietic antigen CD34. However, whether CD34 expression identifies a distinct biologic and/or clinical entity in APL is still a matter of debate. We retrospectively analyzed a series of 107 APL patients observed at a single Institution. In all cases, APL diagnosis was confirmed at the genetic level by RT-PCR amplification of the PML/RARα hybrid. Morphological examination revealed 84 hypergranular and 23 variant (M3v) forms. All patients received uniform treatment with only slight modifications according to the GIMEMA protocols AIDA0493 and AIDA2000. Using a cut-off value of 10% positive staining cells we found a total of 31 patients (29%) positive for CD34 and 23 (21%) positive for CD12; all patients CD2 were also CD34+. Concerning biologic and clinical correlations at diagnosis, we revealed that CD34 expression was associated with the M5 variant subtype (p=0.0001), high type of the PML/RARα transcript (p=0.001), higher WBC counts (p=0.02), high risk category according to Sanz’s score (p=0.013), higher prevalence of the FLT3-ITD mutation (p=0.001) and of disseminated intravascular coagulation (DIC) at presentation (p=0.031). CD34+ also had higher frequency of RAS compared to CD34− patients (p=0.012). As regarding the impact of CD34 expression on treatment outcome no differences were observed regarding the response to induction therapy, whereas a significant difference was seen in the rates of overall survival (p=0.039) and molecular relapse (p=0.002). Our findings suggest that CD34 expression identifies a subset of APL patients with more aggressive clinical features.

**PO-034**

**TAILORED NON AGGRESSIVE CHEMOTHERAPY FOR ELDERLY FRAIL PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)**


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Elderly AML patients (>60yrs) have a poor prognosis because of unfavourable cytogenetics, comorbidities and reduced compliance to therapy. As consequence, treatment strategy should be based on status at diagnosis and the goal of therapeutic choice might be only the overall survival (OS) (Deschler B, De Witte T. 2006; Pellizzari A,M, Drea M., 2007). Aim. To evaluate the impact of non aggressive therapy on OS in a pilot study on elderly AML patients. Patients and Methods. From January 2003 to December 2006, 60 elderly patients with AML-36 male/24 female, median age 71 yr (range 62-88),43 de novo and 17 secondary were treated at our Institution. Of these , 28 pts, all FAB subtypes except M5, were considered frail because of age>80 yrs (11 pts) and/or PS >2 (16 pts). The therapeutic decision was made on the basis of the presence of one or both mentioned parameters. An informed consent document was requested. Cytogenetics were available in 28/1. was classified as favourable, 12 intermediate, 10 unfaevourable. As therapy, patients were treated according to a conservative approach (Group A) and supportive one (Group B) Group A: 18 pts - 6f/10m, median age 75 (range 65-88), PS<2 (15 pts), PS>2 (5 pts), median WBC 2.9x10/L (range 0.8-103) - received, as outpatients, conservative therapy with 6-Thioguanine100 mg/mq/d 5°, two times a month till progression. Group B: 10 pts - 5 M/5 F, median age 71 yr (range 62-88),43 cytogenetics were available in 23:1 was classified as favourable in 17 pts, intermediate 6. and 17 secondary 6. The study is ongoing and, as of March 2007, 20 consecutive AML patients aged less than 65 yrs were included. The induction regimen (FLAI + MY) included Fludarabine (30 mg/sqm), Ara-C (2 g/sqm) on days 1-5, Idarubicin (10 mg/sqm) on days 1,3,5 and Mylotarg (3 mg/sqm) on day 6. Hematopoietic stem cell transplant (HSCT) was planned for all patients in first Complete Remission (CR) after consolidation with high dose Ara-C and Idarubicin (HDAC+IDA). Patients’ outcome and safety were compared with an historical group that received FLAI alone. No significant differences in terms of toxicity or haematologic recovery were observed between the two treatment groups. Conclusions. In elderly AML patients >60 yrs of age, a non-aggressive chemotherapy is feasible and may be considered as an alternative approach in those cases where aggressive treatment could not be safely administered.
a disease which is inherently more resistant to standard cytotoxic agents and to a poor tolerance of chemotherapy due to comorbidity. We evaluated: a) the incidence of unfavourable cytogenetic abnormalities, b) the impact on survival and complete remission (CR) achievement of a conservative therapy vs an intensive one. From January 2003 to December 2006, 49 AML pts-31M/18F, (median age 71 yrs, range 62-88) were referred to our Unit. Thirty-four were de novo AML, 15 were considered secondary-AML (12 to previous myelodysplasia and 3 to solid cancer). At diagnostic, cytogenetics was assessable in 36/49 pts (4 favourable, 19 intermediate, 13 high risk). Five/49 pts died before treatment started; 18/44 were considered frail (median age 72 yrs, range 65-88), and treated as outpatients with a conservative approach including: 6-Thioguanine (6-TG) (100 mg/mq/d days 1°→4°) + AraC (100 mg/mq/d days 5°) twice a month, until progression or death. Twenty-six/44 pts (median age 70 yrs, range 62-74) were enrolled in intensive protocols. Among the 18 pts in conservative therapy, 2 pts (12.5%) achieved CR, 11 pts maintained a stable disease and 5 died early because of leukaemia and/or infections. Among 26 pts treated with intensive therapy: 9 (54.6%) achieved CR, 1 pt partial remission (PR), 7 pts were resistant and 9 died during induction. Median hospitalisation time was 28 days (range 25-60). Median CR was 6.5 months (min 4-max 25). Up to April 2007, 5/18 pts in conservative therapy are alive: 2 CR pts are alive for 10+ and 34+ months, respectively (1m CR and 1 in relapse). The remaining 3 pts, in stable disease, are alive 6+, 12+ and 15+ months respectively. Median overall survival (OS) was 6 months (range 1-34). Up to now, among the 9 CR pts in intensive group, are alive, 2 in CCR (22+ and 17+ months), 1 relapsed. The median OS in this group was 4 months (range 1-40), median OS in CR patients was 16.5 months (range 4-40 months). The overall response rate (ORR) was 25% for the conservative therapy group and 55% for the intensive one. In our study, 15 pts were classified as high cytogenetic risk; among these, only 4 pts are still alive: 1 in CCR (23+ months), 2 in stable disease (6+ and 12+ months), 1 relapsed. Treatment approach should take into account: the inherited disease characteristics, single patient’s comorbidities and psycho-social frailty. The prognosis of elderly AML pts might be improved by a risk-adapted treatment strategy, and by using investigational therapies with the differentiating agents.

PO-036
ALLOGENEIC STEM CELL TRANSPLANTATION IS THE OPTIMAL POST-REMISSION THERAPY IN MINIMAL RESIDUAL DISEASE POSITIVE PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Introduction. Autologous (AuSCT) and allogeneic stem cell transplantation (ASCT) are well established post-remissional strategies for patients with Acute Myeloid Leukemia (AML). However, there is still a debate ongoing about the relative merit of each of these options in first CR. In fact, ASCT for patients in first CR is associated with the lowest relapse rate but also with a higher risk of treatment-related mortality than AuSCT. Therefore, in the attempt to deliver oriented-risk therapies, a number of prognosticators have been identified; among these, minimal residual disease (MRD) promises to be a useful tool. To address this issue, we analysed retrospectively, a series of 142 patients affected with AML in whom flow-cytometry serial determinations of MRD were available.

Methods. The patients were entered into the EORTC/GIMEMA protocols AML10/AML12 (age <61 yrs) or AML13/AML15/AML17 (age=61 yrs), all consisting in intensive induction and consolidation cycles, and, for patients aged <61 years, AuSCT or ASCT. The Maximally Selected Rank Statistic analysis was used to select the MRD level and the time-point of analysis achieving the best prognostic significance in terms of overall survival (OS) and relapse free survival (RFS). This approach confirmed the threshold of 3.5×10^−4 residual leukemic cells after consolidation therapy as a discriminator between MRD+ and MRD− cases with different 5-years OS (60% vs. 18%, p <0.001) and RFS (70% vs 18%, p <0.001). Results. Among the 142 patients under evaluation, we enucleated 55 and 17 who underwent AuSCT and ASCT, respectively. Among the 55 patients submitted to AuSCT, those MRD− had a more favorable outcome than the MRD+ ones, in terms of 5-years OS (66% vs 28%, p=0.006) and RFS (68% vs 14%, p <0.001). In the ASCT group, the low numbers hampered any firm conclusion; however, when the category of post-consolidation MRD+ patients (41) was analysed separately, the use of ASCT was associated with a better RFS than AuSCT (48% vs 14%, p=NS) (Figure 1). We assume the lack of statistical significance merely due to the few cases in the ASCT group.

Conclusions. 1) the threshold of 3.5×10^−4 at post-consolidation check-point is critical to predict outcome; 2) MRD+ patients have an excellent outcome regardless of the post-consolidation therapy; 3) in the MRD+ group, AuSCT seems not to improve the prognosis whereas the use of ASCT is associated with a superior outcome.

Figure 1.

PO-037
INTENSIVE CHEMOTHERAPY INCLUDING AUTOLOGOUS STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA IN HIV+ PATIENTS RECEIVING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY
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Introduction. Clinical outcome of AML in HIV+ pts is poorly documented. Long-lasting responses are rare and optimal treatment is unknown. We report our experience with intensive chemotherapy (CT) including peripheral blood stem cell (PBSC) transplantation in 3 consecutive pts receiving HAART. Patient 1. A 48-year-old HIV+ pt on HAART was diagnosed with AML M4 (46,XY) in 10/01 (CD4=284). After conventional idarubicine (Ida) and Ara-C (92), he obtained CR. After 1 consolidation course he received high dose Ara-C (HDAC) (1 g/mq bid for 4d) + G-CSF 10 mcg/Kg (cycle A) as mobilizing treatment (CD34+ collected 4.5×10^6/Kg, CFU-GM 5.6×10^6/Kg, BU-E 2.2, CFU-GEMM 0.2). On 06/02 he received high dose therapy (HDT) with Thiopetae 10 mg/Kg + Cyclophosphamide 50 mg/Kg for 2 d (TIO-CTX) with stem cell rescue. HAART was continued. No fever, infections or other significant toxicities were seen and engraftment was prompt (N>500 d+10; plt>20.000 d+12). In 07/04 he developed anemia and leuco-monocytosis was diagnosed with CMML (complex cytotype) and in 09/05 (m+47) died by Pseudomonas sepsis. Patient 2. A 35-year-old pt was diagnosed with AML M1 (47,XY,+8) and HIV infection in 05/04. (CD4=132). He was treated on HAART and received induction therapy with doxorubicine+Ara-C + VP-16 (DAV); several infectious complications occurred, including bilateral pneumonia, E.coli sepsis and subcutaneous abscess from MRSE. CyCR was achieved. After a second DAV he received A8 and collected PBSC (CD34+ 6.2×10^6/Kg, CFU-GM 15.9×10^6/Kg, BU-E 15.2, CFU-GEMM 1.6). In 12/04 he received TIO-CTX with stem cell rescue. No significant toxicities were seen and engraftment was prompt (N>5000/mmc d+11; plt>20.000/mmc d+13). In 02/05 a bone marrow (BM) biopsy confirmed CR. At m+9 after PBSCT he developed acute cholecystitis, refused treatment and died. Patient 3. A 55-year-old HIV+ pt, with previous KS (treated with doxorubicine), on HAART, was diagnosed with AML M4 with eosinophilia (46,XY,inv16)
in 06/2006 (CD4=2048). He received induction therapy (Ida+Ara-C+VP-16) and consolidation (Ida+Ara-C), obtaining CyCR. He received mobilizing treatment with PBSC collection. After 12 additional courses with HDAC is alive in CR (m+11).

**PO-038**

**FUNGAL INFECTIONS IN ACUTE MYELOID LEUKAEMIA PATIENTS TREATED WITH INDUCTION REGIMENS INCLUDING FLUDARABINE: A RETROSPECTIVE ANALYSIS OF 224 CASES**

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Infections are an important cause of morbidity and mortality of acute myeloid leukaemia (AML). Invasive Fungal Infections (IFIs) occur in at least 10 to 20% of the patients submitted to induction and consolidation treatments and are responsible for death during induction (DDI) up to the 5% of cases. Furthermore, they may cause a delay in consolidation and intensification therapy with autologous or allogeneic stem cell transplantation and for these reasons they may contribute to increase the relapse rate. Among the risk factors for IFIs it has been included the use of Fludarabine (Fluda), which can induce severe and prolonged immunosuppression. In this study we retrospectively analyzed the infections occurred in 224 newly diagnosed AML patients, aged at least 60 years, consecutively treated with a Fluda containing induction regimen.

One-hundred thirty-five patients were treated with FLAI (fludarabine + cytarabine + idarubicine) and 89 with FLAIE (fludarabine + idarubicine + etoposide). During induction phase, 134/224 (55%) patients experienced a fever of undetermined origin (FUO), the incidence of Gram negative and positive sepsis was 55/224 (24%) and 49/224 (22%), respectively and 4/224 (2%) patients developed a possible/probable IFI. In 9/224 patients (4%) a proven IFI was found (6 Aspergillus and 3 Candida). Taking into consideration the long lasting immunosuppressive effect of Fluda, we collected the data of the incidence of infectious during the first consolidation course (FLAI: n=70; high dose cytarabine: n=65; idarubicine and high dose cytarabine: n=89).

The overall incidence of FUO was 29% (66/224), the number of Gram negative and positive sepsis was 53/224 (24%) and 49/224 (22%), respectively and 4/224 (2%) patients developed a proven IFI (3 Aspergillus and 1 Candida). In all but one case, the fungal infections diagnosed during consolidation occurred in patients who developed an IFI during the previous induction therapy. These data, even though retrospectively collected, suggest that the use of a Fluda-based induction chemotherapy doesn't cause a high number of IFIs, neither during induction, nor during consolidation. In particular, the incidence of infectious complications in our series of AML patients favourably compares to the one reported by other Authors with induction chemotherapy not including Fluda.

**PO-039**

**ROLE OF DASATINIB IN PH-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) AFTER IMATINIB-BASED THERAPY FAILURE: A CASE REPORT**

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Ph+ ALL is largely associated with a dismal prognosis. Imatinib effectively blocks the bcr-abl-related tyrosine kinase activity, and therefore its use is now mandatory in the treatment of this disease. Dasatinib is a more potent abl kinase inhibitor, which appears to be active against 14 out of 15 known imatinib-resistant bcr-abl mutants. In November 2004 Ph+ ALL (p190) was diagnosed in a 60 years old man and induction therapy was soon started according to NII protocol ALL09/2000-STIS71, consisting of association of idarubicin, vincristine, prednisono and asparaginase plus triple-drug intrathecal therapy. Imatinib (600 mg/d) was administered 7 days/month. After 30 days complete remission (CR) was documented at morphologic, cytogenetic and molecular level. The patient was then consolidated with monthly cycles of chemotherapy plus imatinib up to August 2005, and submitted to cranial radio- prophylaxis. No related or matched unrelated donors were available. Starting on September 2005, 2 cycles of high dose chemotherapy with PBSC rescue were therefore administered, the patient being in continuous molecular CR. Maintenance therapy was initiated in December 2005 with daily oral 6-MP and weekly i.m. MTX plus imatinib 400 mg/d, 15 days per month. This therapy was complicated by recurrent grade 4 trilineage hematologic toxicity. In May 2006, 18 months after diagnosis, molecular relapse was documented, followed one month later by cytogenetic (1/100 metaphase Ph+) and four months later by morphologic relapse (10% bone marrow blasts). Leukemic relapse ensued in the context of a severely hypoplastic bone marrow and of patient’s performance status deterioration, thus precluding any further chemotherapy attempt. For these reasons dasatinib therapy (70 mg b.i.d.) was immediately started. No more than Grade 1 side effects were observed, a new morphologic CR was documented after one month therapy and it was confirmed two months later. After 3 months, dasatinib dosage was increased to 100 mg/d, its tolerability continuing to be excellent, a part from transitory and mild diarrhea. This increase of dose enabled a cytogenetic remission to be obtained on January 2007. On May 2007, that is 8+ months from dasatinib start, the patient remains in continuous cytogenetic remission. Figure 1 illustrates serial evaluation of MRD by means of real time PCR and documents the effectiveness of dasatinib-driven clearance of bcr/abl positive cells in this patient.

![Figure 1](image-url)
**PO-040**

**TREATMENT DECISION-MAKING FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA: SINGLE CENTER EXPERIENCE**

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**Introduction.** Currrently no clear indications exist on the role of intensive chemotherapy (IC) in the treatment of elderly patients affected by acute myeloid leukemia (AML). The aim of the study was to investigate the efficacy and safety of IC in a subset of AML elderly patients.

**Methods.** Seventy-one patients aged 60 years or older with AML were consecutively admitted between 2000 and 2007 and were considered suitable for the analysis. Thirty-four patients with ECOG performance status (PS) ≤ 2 and age ≥ 70 years were treated with IC (daunorubicin, ARA-C, etoposide 3+7+5; FLAG-Ida: Group A). Thirty-two patients aged > 70 years and/or PS > 2 were treated with non curative approaches (Group B). Five patients aged between 70 and 75 years with a PS ≤ 1 were treated with IC. The mean age of the patients was 69.2 (60-85), the mean age of Group A and Group B was 68.3 (60-78) and 73.7 (65-85), respectively. The median follow-up was 7.9 months. Seven of 39 group A and nine out of 32 group B patients showed active infection at diagnosis. Nineteen group A and 14 group B patients showed comorbidity (diabetes, ischemic heart disease, previous neoplasia, hypertensión). A normal karyotype was detected in 12 Group A and in 9 Group B patients, and abnormal karyotype was recorded in 12 Group A and 7 Group B patients. In 15 patients in both arms, cytogenetics was not evaluable. The median survival of Group A and Group B was 9.4 and 5.8 months, respectively (Log rank test = 5.3, p < 0.05). Induction mortality was 8% (3 patients age 65, 68 and 69 years). The complete remission rate (CR) was 43%. Fifteen patients completed the induction and consolidation therapy, while 2 pts in RC after induction discontinued IC for toxicity (one for pneumonia and persistent pancytopenia and one for lung failure). The median survival of patients in CR and of non responsive patients was 27.3 and 6.7 months, respectively (Log rank test = 13.1829, p = 0.001). The mean DFS for responding patients was 18 months, median not reached. A significant difference in comorbidity was recorded for CR versus non CR patients: 23% versus 68%, respectively while unfavourable cytogenetics and infections were comparable in both arms (55% vs 27%, and 11% vs. 18%, respectively). **Conclusions.** With our therapeutic strategy we were able to single-out a subset of elderly patient with a significant survival benefit with IC. Elderly patients who would benefit from intensive chemotherapy need a good PS and no comorbidities. These data were supported from the low induction mortality and the overall RC rate. However a comprehensive geriatric assessment (including evaluation comorbidities) is raccsomended to be incorporated into clinical analysis for assigning regimen intensity.

**PO-041**

**FATAL OVERWHELMING ACUTE PROMYELOCYTIC LEUKEMIA (APL), MICROGRANULAR VARIANT (M3v), WITH MASSIVE EXTRAMEDULLARY DISEASE AND UNCHARACTERISTIC IMMUNOPHENOTYPE: A CASE REPORT**

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The microgranular variant (M3v) of acute promyelocytic leukemia (APL) accounts for approximately 20% of all cases of APL. M3v immunophenotype is more heterogeneous than classic APL, with variable expression of CD2, CD34 and HLA DR. We report an unusual case of APL, microgranular variant (M3v), with CD64, CD13, CD14 expression, characterized by an aggressive and diffuse extramedullary disease and overwhelming fatal evolution early during induction treatment. A 38-years-old male presented with dyspnoea on exertion, cough, fever and leukocytosis. Performance status was 2 according with WHO scale. Clinical examination revealed epatosplenomegaly. At admission blood cell count was: Hb 9.7 grams/deciliter, WBC 30×10^9/Liter, N 53%, E 47%, PLT 24×10^9/Liter, AST 271 Unit/Liter, ALT 413 Unit/Liter, transaminases 100 times normal values, creatinine 2.3 milligrams/deciliter, LDH 1513 Unit/Liter, AST and ALT 308 and 360 Unit/Liter, respectively. A blast population representing 99% of bone marrow nucleated hematopoietic cells and 90% of the peripheral blood cells was identified. Blasts had typically bilobed or reniform nuclei with inconspicuous cytoplasmic granules and rare Auer rods but showed intense reactivity with myeloperoxidase by cytochemical staining while nonspecific esterase was negative. Immunophenotyping was performed on peripheral blood cells. Positivity for CD2 (51%), CD34 (86%), CD38 (80%), CD64 (85%), CD13 (94%), CD45 (99%), HLA DR (35%), CD33 (96%), CD13 (94%) and CD117 (93.5%) was recorded. CD64 expression displayed a light scatter pattern indistinguishable from that of AML with monocytic differentiation; however, coexpression with CD14 was not detected. Cytogenetics of 96/100 cells (GTG banding) from 24-hour and 48-hour unstimulated bone marrow cultures showed 46, XY, t(15;17), confirmed by FISH. At diagnosis, RT-PCR analysis for bcr3 PML/RAR alpha transcript was positive only in bone marrow specimen. Induction regimen was rapidly started according to the current guidelines, with administration of adriamycin (45 milligrams/kg body weight, po) and idarubicin (12 milligrams/kg body weight, iv; only one dose was administered). On day +3 patient developed a rapid clinical worsening with fever, abdominal pain and serious dyspnoea. Abdomen CT scan revealed massive enlargement of liver and spleen while chest CT showed thoracic adenomegaly and pleural effusion. White blood cells raised to 98×10^9/Liter, associated with disseminated intravascular coagulation and serious hepatic and renal insufficiency (LDH 9650 Unit/Liter, transaminases 100 times normal values, creatinine 2.5 milligrams/deciliter). Patient died by multi-organ failure on day +4. Post mortem examination revealed massive leukemic involvement of lungs, heart, kidneys, spleen, bone marrow and brain. Positivity for bcr3 transcript was confirmed in bone marrow post-mortem specimen. The diagnosis of M3v can be difficult because the morphologic, cytochemical and immunophenotypic features often are non specific. The significance of CD34 expression is unknown but it likely identifies an immature form of APL; on the other hand, CD2 expression is correlated significantly with leukocytosis, a key negative prognostic indicator demonstrated in many studies. In this case, a retinoic acid syndrome appeared unlikely because of the dose reduction applied, the rapid onset and the results of post-mortem examination, showing diffuse infiltration of leukemic cells. Considering the massive extramedullary involvement, a rare event in APL at diagnosis but described at relapse, we can speculate about a very aggressive disease for which a different treatment is probably necessary. The present case emphasizes the need of further studies in APL patients, to assess whether a heterogeneous immunophenotypic pattern could have a prognostic impact.
PO-042
FOUR-DRUGS INDUCTION INTENSIFIED REGIMENS (IDARUBICIN, CYTARABINE AND ETOPOSIDE PLUS FLUDARABINE OR MYLOTARG - FLAIE AND MYAE) FOR ACUTE MYELOID LEUKEMIA. RESULTS OF A PILOT STUDY
Paolini S,1 Piccaluga PP,1 Rondoni M,1 Malagola M,2 Papayannidis C,1 Laterza C,1 Verlicchi L,1 De Rosa E,1 Ottaviani E,1 Iacobucci I,1 Russo D,1 Canioni A,1 Fanin R,1 Visani G,1 Baccarani M,1 Martinelli G1 Institute of Hematology and Medical Oncology L. and A. Seràgnoli, University of Bologna; 2Chair of Hematology and Unit of Blood Diseases and Cell Therapy, University of Brescia; 3Chair of Hematology, University of Udine; 4Department of Hematology, S. Salvatore Hospital, Pesaro, Italy

Background. Though the standard induction treatment in acute myeloid leukemia (AML) patients aged ≥60 years is still based on the classical combination of antracycline and cytarabine (3/7 scheme and similar), intensified regimens seemed to be superior at least in non-randomized trials. Recently we showed that FLAIE (fludarabine, arabinosyl cytosine, idarubicin) is at least as effective as the conventional ICE (idarubicin, cytarabine, etoposide). Aim of the study. We evaluated the safety profile and the efficacy of two four-drugs induction regimens adding either fludarabine (25 mg/m² days 1-5) or mylotarg (5 mg/m² day 6) to idarubicin (6 mg/m² days 1-5), cytarabine (1 g/m² days 1-5), etoposide (100 mg/m² days 1-5) (FLAIE and MYAE, respectively).

Methods. Sixty-six consecutive AML patients were enrolled either in the FLAIE (N=44, from 2002 to 2005) or in the MyAE (N=22, from 2005 to 04/2007) regimen. The main clinical and biological characteristics were definitely comparable. The median age was 45 and 48 years, respectively. Seventy and sixty-four percent of cases, respectively, were considered at high risk basing on karyotype, WBC count, and FLT3 status. Consensus induction therapy consisted of 3 cycles of ID-AraC and Ida. Results. The complete remission (CR) rate was 75% and 59% for FLAIE and MYAE, respectively (p=NS). Death during treatment rates were 5% and 0%. After 1 consolidation course the overall CR rate was 80% and 73%, respectively. After a median follow-up of 27 months (1-62), 41% of patients are alive in CR in the FLAIE group (12 SCT and 3 ASCCT). Seventy-seven percent of patients are alive in CR in the MyAE group after a median observation of 5.5 months (1-25). Toxicity was comparable in the two regimens. The median time to ANC recovery (>1.0×10⁹/L) was 31 and 23 days for FLAIE and MYAE, respectively. The median time to PLT recovery (>100×10⁹/L) was 28 and 24 days, respectively. The median number of neutropenic fever episodes for patients was 1 and 1.4 in the 2 groups, respectively. Grade III/IV G1 toxicities occurred in 11% and 22% of cases, respectively. Conclusions. This preliminary analysis showed that four-drugs intensified induction therapy is feasible in AML patients aged ≥60 years. Nevertheless, when compared with previously tested ICE and FLAIE regimens, these schedules seemed to be not more effective and with similar toxicities profiles. Future analyses and randomized trials will define their possible role in AML treatment. Acknowledgement. Supported by: European LeukemiaNet, COFIN 2008, AIRC, AIL.

PO-044
FLT3 POSITIVE ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

Introduction. Mutations of FMS-like tyrosine kinase 3 (FLT3) have been detected in about 30% of adult patients with acute myeloid leukemia (AML). The alterations most often involve small tandem duplications of amino acids within the juxtamembrane domain of the receptor (ITDs) or point mutations of Asp835 in the kinase-loop (D835). The aim of our study was to evaluate the prognostic significance of ITDs and D835, and the role of transplant procedures in FLT3 positive AML patients. Methods. The study includes 167 adult AML patients, with the exclusion of M3, referred to our institution and entered into the GIMEMA/AML12 trial since January 1999. One hundred and thirty two patients (79%) were wild type (WT) for the FLT3 gene, 25 (15%) presented ITDs, 8 (8%) D835 and 2 a lack of the two alleles. Results. The complete remission (CR) rate was 75% and 59% for WT and D835 positive patients, respectively (p=0.0001). Even the 5 year disease-free survival (DFS) of the ITD positive patients is significantly lower for ITD positive patients, 68%, compared to 86% for WT patients (p=0.03), while all D835 patients achieved a CR. Forty-eight of the 113 WT patients in CR after induction received an autograft, 24/113 an allotransplant and 6 did not undergo a transplant procedure due to early relapse (n=6), death (n=10) or ineligibility (n=28). Sixty-one are in continuous CR (CCR). Regarding the 17 ITD positive patients in CR after induction, 10 underwent an autotransplant, 3 an allotransplant and 4 did not undergo a transplant procedure due to early relapse (n=2) or ineligibility (n=4). All autografted patients are in CCR. With regard to the 8 patients with D835, 3 received an autotransplant, 2 an allotransplant and 3 did not receive a transplant due to ineligibility. Four patients remain in CCR, regardless of the postremission therapy. The 5 year overall survival (OS) of the patients with ITD is significantly lower compared with that of WT and D835 positive patients (24% vs 49% vs 50%, respectively, p=0.005). The 5 year disease-free survival (DFS) of the ITD positive patients is significantly lower (23% vs 50% vs 50%, respectively, p=0.01). The 5 year EFS for ITD, WT and D835 positive patients is 16% vs 44% vs 50%, respectively (p=0.0007). Conclusions. Our study confirms the negative prognostic impact of ITDs especially in patients who do not undergo a transplant procedure. There is no evidences that D835 influences both CR achievement and survival as compared to WT patients. Prospective trials with the new tyrosine kinase inhibitors are warranted, particularly for ITD positive patients.
PO-045
PROGNOSTIC IMPACT OF HYPERLEUKOCYTOSIS IN ADULT ACUTE MYELOID LEUKEMIA: A SINGLE-CENTRE RETROSPECTIVE STUDY
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Introduction. The prognostic impact of leukocytosis in acute myeloid leukemia (AML) is not clearly defined. Hyperleukocytic AML (WBC count above 100×10^9/L) reported in literature is about 10% of all AML and is considered to have a poor prognosis due to high early death rate secondary to leukostasis. The prognostic impact of hyperleukocytosis on the outcome has been the object of few studies in adults. Materials and methods. The clinical characteristics and outcome of 45 consecutive adult patients with newly AML presenting to our division from 1995 to 2005 with WBC count above 100×10^9/L were reviewed (Table 1).

Table 1. Hyperleukocytic AML

<table>
<thead>
<tr>
<th>Characteristics at presentation</th>
<th>Cohort (45 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>56 (range 17-80)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>23/22</td>
</tr>
<tr>
<td>Median WBC (100×10^9/L)</td>
<td>159</td>
</tr>
<tr>
<td>Median platelet count (100×10^9/L)</td>
<td>1486</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>9</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>1</td>
</tr>
<tr>
<td>Basophils</td>
<td>1</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>65 (range 25-80)</td>
</tr>
<tr>
<td>TAE</td>
<td>0.074</td>
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</tbody>
</table>

PO-046
TREATMENT OF ACUTE MYELOGENOUS LEUKEMIA WITH VALPORIC ACID IN COMBINATION WITH LOW-DOSE ARA-C IN ELDERLY PATIENTS
Corsetti MT, Salvi F, Perticone S, Allione B, Baraldi A, Bellora A, De Failli L, Gatto S, Pietrasanta D, Primon V, Levis A
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Clinical therapy of patients with acute myelogenous leukemia (AML) who cannot undergo classical induction therapy because of age or comorbidity is limited to support and cytoterapy. Valproic acid (VPA) has been shown to inhibit histone deacetylase activity and to synergize with Ara-C in the differentiation induction of AML blasts in vitro. Methods. We treated 13 patients with AML with VPA therapy (serum concentrations 50-100 microg/mL) and cycles of Ara-C (40 mg/day for 8 to 10 days every month). Eight patients had AML, 3 pts had RAEB and the last 2 pts had blastic evolution of Myelomonocytic Leukemia and Essential thrombocytemia. Median age was 68 yrs (range 64-74). Two pts started therapy at diagnosis, 3 were resistant after induction chemotherapy, 7 pts were relapsed after complete remission from a first induction and one after allogeneic transplant. All patients were excluded from conventional chemotherapy for age and multiple comorbidities, with seven of them defined frail patients because of 3 concomitant medical conditions requiring therapy. Evaluated for autosufficiency with ADL score, 2 pts did not reach the score 6, while in functional autonomy with IADL score, 7 did not reach the score of 8. Results. Three patients received the treatment for 6 cycles, the other patients had a median of 2 cycles (range 1 to 4). Hematological toxicity requiring platelet transfusion was seen in 8 patients, while 3 pts required red cell transfusion. Transient hyperammonemia was seen in one patient; liver toxicity one patient. Median overall survival was 4 months (range 1 to 12). The three patient with 6 cycles achieved complete response. In the other ten patients, one of them became independent from red cell transfusion and one from platelet transfusion. In complete responders, median response duration was 6 months. One patient, still in remission after 12 months, one patient underwent allogeneic transplantation with reduced intensity conditioning and one relapsed after six months. Of the other 10 pts who did not achieve complete response, 8 died for progression at a median of 2 months from the start of therapy (range 1-7 months); 2 are still alive after 8 and 7 months. Discussion. Adding VPA to low-dose Ara-C, some complete responses and some improvement in peripheral cytopenias can be achieved without a significant increase in toxicity. Therefore this therapeutic protocol is feasible also for patients who cannot have aggressive therapy.

Table 2. Response to treatment according to WBC (100x10^9/L)

<table>
<thead>
<tr>
<th>Response to treatment</th>
<th>WBC &gt;100</th>
<th>&lt;100</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° patients</td>
<td>45</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>CR (%)</td>
<td>15 (33%)</td>
<td>129 (64%)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Early deaths</td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>OS (months)</td>
<td>10</td>
<td>19.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

PO-047
EVALUATION OF FMS LIKE TYROSINE KINASE 3 (FLT3) INTERNAL TANDEM DUPLICATION AND D835 MUTATION EXON 17 IN ACUTE MYELOID LEUKEMIA AND ACUTE PROMYELOCYTIC LEUKEMIA
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Introduction. We have studied prognostic values of FLT3 (FMS Like Kinasi 3) gene, and ex on 17 mutations in acute myeloid leukemia. Internal tandem duplications have been described in 35-40% of patients with acute myeloid leukemias. Aspartic acid (asp) substitution D835 on exon 17 mutation of FMS Like Kinasi 3 gene and internal tandem duplications were evidenced. Objective. FLT3/ITD and D835 mutations study in acute myeloid leukemias in Cancer Hospital of Cagliari. Material. From January 2003 to May 2007, 72 new diagnosis of acute myeloid leukemia (AML) have been evaluated. All patients were adults, (range 25-80). Acute myeloid leukemias were 54 (75%) and 18 (25%) were acute promyelocytic leukemias. Methods. RNA and DNA from peripheral blood and bone marrow have been utilized. All cases were evaluated
with PCR procedure, 4 microliter of RNA was retro transcribed in CDNA and amplified with 50 picomoles of each specific primers (forward and reverse) and reverse transcriptase. Annealing temperature was set at 52°C and the extension time was 2 min. Agarose gel electrophoresis, 100 nanograms of DNA were evaluted with PCR procedure for D853. Results. In 5.5% cases of acute myeloid leukemia (AML) FLT3/ITD transcript were positive while in 16.6% cases of acute promyelocytic leukemia (APL) FLT3/ITD transcript were positive. In 10% cases of acute myeloid leukemia (AML) D835 and in 7.7% cases of acute promyelocytic leukemia (APL) D835 transcripts were positive.

PO-048

SIX-YEARS RETROSPECTIVE MONOCENTRIC EXPERIENCE (2000-2006) OF ACUTE MYELOGENOUS LEUKEMIA IN THE ELDERLY

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More than 50% of acute myeloid leukemia (AML) cases occur over 60 years-of-age. As a matter of fact, a high incidence of treatment-relat ed morbidity and adverse biological features such as antecedent hemato logical disorders and high risk karyotype, has been advocated to account for a poorer outcome in the elderly. This retrospective monocentric study was undertaken to analyse the survival pattern and to evaluate the feasibility of chemotherapy, according to comorbidity, of 128 consecutive unselected elderly AML non M3 patients, observed from January 2000 to October 2006 in our institution. We have also investigated possible prognostic differences between de novo AML and AML following antecedent hematological disorders (sAML) and between cytogentic subgroups (low-intermediate versus high risk). Eight patients were excluded from statistical analysis because of dismal clinical conditions and incomplete staging of disease. One-hundred-twenty newly diagnosed and previously untreated AML patients were considered; 65 were male and 55 female, median age was 74 (61-88); 73 cases of acute promyelocytic leukemia (APL) FLT3/ITD transcript were positive. In 10% cases of acute myeloid leukemia (AML) FLT3/ITD and in 7.7% cases of acute promyelocytic leukemia (APL) D835 transcripts were positive.

PO-049

POST-REMISSION MAINTENANCE TREATMENT WITH LOW-DOSE CHEMOTHERAPY + DIFFERENTIATING AGENTS CAN PROLONG REMISSION DURATION AND SURVIVAL IN POOR RISK AML AND MDS PATIENTS

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Acute myeloid leukemia (AML) in patients over the age of 60, as well as high risk myelodysplastic syndromes (MDS) carry a very poor prognosis, with median CR duration, after intensive chemotherapy, ranging between 6 and 12 months (Jehn U et al: Leukemia 20: 1722; 2006). We used a combination of low-dose chemotherapy + 13-cis retinoic acid (RA) and dihydroxlated vitamin D3 (D3) as a maintenance treatment in 30 AML (either ≥ 60 year old and/or with secondary/ therapy-related disease) and 10 high risk MDS patients, in first CR after different schemes of anti-AML chemotherapy and unsuitable to allogeneic transplant. Median age was 65 (55-76). Cytotypic analysis, successfully performed in 81/40 patients, revealed a normal cytotype in 17 and chromosome abnormalities (except chromosome translocations) in 14. Thirty-two patients had received consolidation chemotherapy, that in 3 cases included autologous stem cell transplantation. Maintenance started after a median of 2.5 months CR and was continued until relapse or 3.5-4 years of CR. All patients received a combination of RA (20-40 mg/day) + D3 (1 microgram/day), associated in 37 patients to intermittent courses of 6-thioguanine (40 mg/day for 3/5 weeks). In 29 patients a two week course of low dose ARA-C (5 mg/m2 x 2/ day) + 6-mercaptopurine (50 mg/day) was substituted for a 6-thioguanine course every 10-15 weeks. Two patients relapsed before starting the maintenance and were evaluated on intention to treat basis. Two patients only stopped the treatment for poor tolerance after one month. In all other patients most common side effects were retinoid-induced lip/ skin dryness and grade 2-3 thrombocytopenia after ARA-C courses. A few patients required occasional platelet transfusions whereas neutropenia was mild. After a median follow up of 22 months for alive patients, median CR duration reached 20 months, with 39%, 33%, 29%, 17% of patients projected in CR at 2, 3, 4, 5 years, respectively, from CR achievement. Median survival was 25 months, with 43%, 33%, 29%, 20% projected alive at 2, 3, 4, 5 years, respectively, from diagnosis. Diagnosis (AML vs. MDS) did not influence the outcome, conversely CR was significantly longer in patient with normal cytotype: median 43 months vs. 6.5 months for those with chromosome abnormalities (p: 0.05-0.0025). Our protocol of maintenance therapy was feasible and prolonged CR duration, compared to literature data, in poor prognosis AML/MDS patients.

PO-050

SIGNIFICANCE OF DEL 18 IN ACUTE PROMYELOCYTIC LEUKEMIA FOLLOWED BY A LYMPHOPROLIFERATIVE DISORDER (B-CLL)

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1Division of Haematology, Azienda OspedalieraBianchi-Melacrino-Morelli Reggio Calabria; 2Operative Unity of Medical Genetics

Acute promyelocytic leukaemia (APL) is a biological and clinical subtype of acute nonlymphocytic leukaemia characterised by translocation t(15-17) with molecular expression of PML-RARα. We describe a patient with APL and del 18p. Six months prior to APL diagnosis the patient received alpha interferon plus ribavirin for HCV infection. At diagnosis he presented leukaemia and thrombocytopenia; marrow blasts immunological characterization showed: CD9 65%; CD117 55%; HLA-DR 15%; and/or supportive care. The optimal management of acute myeloid leukaemia in patient over 80 years-of-age is still matter of debate. Our results encourage the use of stratification system based on specific geriatric evaluation, validated comorbidity scales and cytogenetics, for the appropriate selection of patients fit for aggressive therapies. Moreover, patients with unfavorable karyotypes and low comorbidity scores seem to be candidates for novel investigational trials. For unfit patients, it remains to demonstrate which is the best supportive care.
CD45 90%; CYMPO 85%. The t(15-17) was detectable by FISH in about 50% of blasts. Deletion of 18p was present in 30% while the somatic cells didn't show any deletion. The patient started chemotherapy according to protocol AIDA: 2000 GIMEMA, intermediate risk, and obtained complete remission (CR) 35 days after induction therapy. After consolidation therapy t(15-17), PML-RAR-α and del 18 were negative. During maintenance therapy with ATRA PML-RAR-α remained negative while the clone with del18 increased up to the 60% (of cells) while there was no evidence of APL relapse into the bone marrow. After two years of the end of therapy for maintenance, the patient developed a lymphoproliferative disorder (B-CLL). Del 18p is an unusual in this setting. In HCV-associated lymphoproliferative disorders there is chromosome 18 impairment as only t(14,18). In these cases antiviral therapy may induce regression of t(14,18) bearing B-cell clones. However in mouse models ribavirin is mutagenic in bone marrow cells. In this case we might speculate that the two disorders could be concomitant and that the observed additional cytogenetic anomaly (del 18p) could be associated with the development of the lymphoproliferative disorder and that conventional chemotherapy for APL might have delayed the onset of the BCLL disorder.

PO-051
FLAG-IDA IN THE TREATMENT OF REFRACTORY/RELAPSED ACUTE LEUKEMIA: SINGLE CENTER EXPERIENCE
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Relapsed or refractory adult acute myeloid leukemia (AML) have a poor prognosis. The strategy for treating this patients is through reinduction chemotherapy followed by allogeneic stem cell transplantation, provided that the toxicity of the salvage regimen is acceptable. High or intermediate dose cytarabine has been reported to be effective in the salvage treatment of AML; addition of the purine analogue fludarabine to cytarabine increases the rate of accumulation of cytarabine in leukemia blasts and the response to chemotherapy may be improved by addition of idarubicin, an anthracycline that is less susceptible to multidrug resistance. Based on these considerations, we evaluated the efficacy and the toxicity of FLAG-IDA in a series of 80 refractory/relapsed AML. Fifty-four patients (67.5%) were in first relapse and 35 female with a median age of 43 years (range 15-63). All patients received autologous stem cell transplantation, 9 were judged in remission, 19 received allogeneic stem cell transplantation, 8 patients achieved CR, 35 days after induction therapy. After consolidation therapy t(15-17), PML-RAR-α and del 18 were negative. During

PO-052
PROGNOSTIC ROLE OF IMMUNOPHENOTYPING IN ACUTE MYELOID LEUKEMIA AND HIGH RISK MYELODYSPLASTIC SYNDROMES
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Background. Cytogenetic alterations are the most important prognostic factors in Acute Myeloid Leukemia and in Myelodysplastic Syndromes. However it’s difficult to have information concerning chromosomal abnormalities from all patients. Immunophenotyping is a major tool to assign leukaemia blast cells to myeloid lineage. The prognostic value of immunophenotyping in AML and in MDS with excess of blasts (MDS-EB) is not well defined. Therefore we analyzed the pattern of expression of selected antigens CD13, CD15, CD33, CD34, CD117, DR, CD14, CD64 in bone marrow blasts of de novo AML (except FAB M3) and MDS-EB WHO II by flow cytometry. The blast cells were identified according their localization on CD45/SSC dot plots. Aims. The aim of the study was to verify the relationship between antigen expression, FAB subtype and outcome (remission rate). Methods. We analysed 51 patients (20 female, 31 male); 42 with AML (1 FAB MO, 10 FAB M1, 7 FAB M2, 15 FAB M4, 7 FAB M5, 2 FAB M6) and 9 with AEB WHO type II treated in our department from January 2003 to December 2006. The median age was 56 years (range 17-83 years). All patients received standard myeloablative induction chemotherapy for AML. The responses rates at induction therapy were: 57% complete remission (CR); 19% partial remission (PR); 5% resistance (R) and 19% of the patients died in aplasia. The analysis showed that the patients with AML blasts negative for CD13 and CD34 and at the same time positive for CD3 and CD64, had a higher complete remission rate (57%), independently from FAB subtype risk. This antigen expression was confirmed in patients with normal karyotype, age < 60 years and normal value of serum LDH. The AML blasts positive for CD13, CD33, CD34, CD117 were related with poor outcome of disease (9 partial remission and 3 resistance) according to FAB risk. Four of these cases with de novo AML showed chromosomal abnormalities: 46xy (6) 45xy (6) (p22;23)(4); 46xy(14) 47xy (4) (11); 46xx (t;5) (9;22); 44xx-,5-,7,-,dic(17)(12) q10,11, s(12), p40,+8,-12,-17,-17,+,+mark-er/46xx. The HLA DR positivity appear non specific. The expression of CD19 was seen in 1 patient with AML FAB M1 and biomolecular study was positive for MLL rearrangement. Conclusions. These data show the prognostic relevance of immunophenotype in AML and in AEB patients and may be useful for risk stratification in these patients. Nevertheless it is necessary to analyze a larger number of cases for stratification and to include the immunophenotype as individual prognostic factor and it could be used for a new classification of AML.

PO-053
COMPLEX KARYOTYPIC ALTERATIONS AND IMMUNOPHENOTYPING IN DE NOVO ADULT ACUTE MYELOID LEUKEMIAS (AML)
Improtta S, Carola A, Lucania A, Quirino AA, Sagristani M, Villa MR, Tommasino C,1 Mastrullo L
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In adult AML the complex karyotypic alterations at the diagnosis characterize a sub-group of patients with worse prognosis. It is also known that the complete immunophenotypic characterization contributes to define the prognosis of de novo adult AML. We have investigated immunophenotypic pattern of leukemic blasts in a group of adult de novo AML with complex chromosomal anomalies. We have observed 17 young adult de novo AML patients (median age: 41 years, r: 27-55) in the last four years, who presented complex chromosomal anomalies in bone marrow blood. On the basis of FAB classification the patients were considered LMA-M5 (5 pts.), LMA-M2 (4 pts.), LMA-M4 (4 pts.), LMA-M1 (3 pts.) and LMA-M0 (1 pt.). Four patients showed internal tandem duplications of FLT3, eleven patients presented hyperleukocytosis (WBC> 40x10^9/L). The clinical outcome was that one of a high risk AML; at the present only two patients are still alive in CR (+21 months and +27 months). We found at the diagnosis in all the cases the same immunophenotypic pattern: CD34+, CD38low/+, CD13+, HLA-DR+. Moreover 15 out of 17 samples showed CD11b expression, and 13 out of 17 were CD13 negative. Several studies have shown the prognostic significance of the expression of differentiation myeloid markers at the diagnosis of adult
de novo AML. However, specific immunophenotype expression patterns associated with complex chromosomal anomalies are still unknown. Further studies are warranted to confirm the correlation between complex chromosomal anomalies and CD34+, CD38high, CD33+, HLA-DR-immunophenotypic pattern.

PO-054
THE REALISK OF THERAPY RELATED LEUKAEMIA (TRAL) IN MULTIPLE SCLEROSIS (MS) PATIENTS TREATED WITH MITOXANTRONE (MITO): AN OPEN DEBATE
Cereda M,1 Chiari,chini A,1 Bartolini M,1 Dongaronzo V,1 Ronci B,1 Anacleterico B,1 Anticoli P,1 Bellantonio P,1 Gaserini C,1 Ruggeri S,2 Cantore GP,1 Annino L1
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Mitoxantrone, a DNA-topoisomerase II inhibitor, has shown to be able to reduce the progression of neurological disability and relapse frequency in Pts with active forms of MS (secondary-progressive, progressive-relapsing, worsening relapsing-remitting disease). In MS Pts the use of MITO as single drug may be associated to an increased risk of TRAL. We report two further cases of secondary acute promyelocytic leukemia (s-APL) occurring in Pts previously treated with MITO for progressive MS: 1) a 55 year-old man admitted in November 2004 to our Department because of aplastic anemia. He had had MS since 1997 and, from May 2002 to September 2004, was given MITO for a total dosage of 80 mg (8 doses of 8mg/m2 every three months). He died because of cerebral haemorrhage before starting ATRA treatment. The interval between last MITO dose and sAPL was 7 months. 2) a 27 year-old man transferred to our Department because of cerebral haemorrhage in February 2006. The patient had had MS since 1999. In PB smear atypical hyper granular promyelocytes and PML/RAR alpha fusion gene were present, thus a diagnosis of sAPL was made. Pts died soon after the hospitalization; he had been treated with MITO from September 2004 to July 2005 (8mg/m2 monthly doses, total dosage of 100 mg). The interval between last MITO dose and sAPL was 7 months. The incidence of TRAL in our cohort of Pts is 0.71% (2 cases/280 Pts). It is noteworthy that, including our Pts, 10/15(66%) of all reported cases in literature are sAPL, a FAB subtype rarely observed as TRAL after other chemotherapy regimens. This high percentage of sAPL post MITO treatment and recent experimental evidences (Mistry AR et al NEJM 2005) suggest that MITO could be directly involved in inducing the t(15;17)/PML-RAR rearrangement which is the trigger for leukaemia. Some important main questions are, at present still to be debated: 1) the real incidence of TRAL in MS Pts undergoing MITO treatment is not well yet established. The reported rate ranging from 0.07 to 0.8%; 2) up to now it is not well recognized if TRAL occurrence is directly related either to total MITO dosage administered, schedule timing (monthly vs every three month doses) and treatment length. TRAL might represents a fatal event, thus the risk of TRAL development must be carefully evaluated before enrolling MS Pts in therapeutic program including MITO. A valid appraisal of TRAL incidence will require a longer follow-up in more patients. The optimal regimen and dose for MITO therapy in MS remains an open debate.

PO-055
THE ROLE OF GEMTUZUMAB OZOGAMICIN IN ELDERLY AML PATIENTS IN COMPLETE REMISSION
Quintini G, Caramazza D, Barbera V, Maisano S, Piazza G, Spadola V, Di Trapani R and Abbaddessa V
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Acute myeloid leukemia (AML) is a disease of the elderly. The majority of patients are indeed diagnosed in their 6th and 7th decade of life. AML in elderly patients is associated with poor response to conventional chemotherapy and limited long-term survival. Such poor outcome reflects a higher incidence of abnormal leukaemia cell cytogenetics and multidrug resistance mechanisms (such as P-glycoprotein-mediated drug efflux), a low bone marrow reserve which likely prevents or delays the recovery of haematopoiesis after treatment, and the occurrence of co-morbidities. Gemtuzumab ozogamicin (GO) is an immunonjugate with a humanized anti-CD33 that after internalisation, releases a cytotoxic drug, calicheamicin. More than 80% of AML patients have myeloid blast cells that express the CD33 surface antigen. GO as a single agent has low antileukaemic activity (Sievers et al., 2001). However, GO is being used at lower doses in combined chemotherapy regimens as induction or postremission therapy. Limited studies have been conducted so far but results appear encouraging and phase III trials are ongoing. We report here our experience with 11 AML patients (Table 1) with a median age of 72.2 years. Patients were consecutively and were treated between May 2004 and May 2007 with standard induction protocols.

Table 1. Patients’ Characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>72.2 (median)</td>
</tr>
<tr>
<td>CD33+ blasts (%)</td>
<td>92.1 (median)</td>
</tr>
<tr>
<td>Performance status</td>
<td>76-83 (range)</td>
</tr>
<tr>
<td>#</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>AML de novo</td>
<td>5</td>
</tr>
<tr>
<td>Secondary AML</td>
<td>0</td>
</tr>
<tr>
<td>AML developed from MDS</td>
<td>5</td>
</tr>
<tr>
<td>AML in 1st relapse</td>
<td>1</td>
</tr>
<tr>
<td>Induction regimen</td>
<td></td>
</tr>
<tr>
<td>Fludara (FLAG)</td>
<td>9</td>
</tr>
<tr>
<td>Non Fludara (7+10)</td>
<td>2</td>
</tr>
<tr>
<td>Time from MDS to AML (months)</td>
<td></td>
</tr>
<tr>
<td>&lt; 12</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 12</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
</tr>
<tr>
<td>Cytogenetic groups</td>
<td></td>
</tr>
<tr>
<td>Poor risk</td>
<td>4</td>
</tr>
<tr>
<td>Favourable risk</td>
<td>3</td>
</tr>
<tr>
<td>Not tested</td>
<td>4</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>CR rate</td>
<td>2</td>
</tr>
<tr>
<td>DFS (months)</td>
<td>9.4 (median)</td>
</tr>
</tbody>
</table>

These included Fluda (FLAG, 9 patients) or non-Fluda induction regimens (daunorubicin+Ara-C in 1 patient, and idarubicin+Ara-C+etoposide in 1 patient). After 1 or 2 induction courses, two postremission courses were given. Postremission MPAC regimen consisted of fludarabine 15 mg/m2/bid i.v. on days 2-4, Ara-C 0.5 gm/m2/bid i.v. on days 2-4, G 4.5 mg/m2/d.i.v. on day 1 with the addition of cyclosporin A 6 mg/kg bw i.v. followed by 16 mg/kg bw on days 1-2. Among 7 patients with available cytogenetic data at the time of diagnosis, 4 were in the poor-risk group and 3 were in the favourable-risk group. Patients were determined to have CD33-positive AML by analysis of bone marrow aspirates and by immunophenotyping. The rate of clinical remission (CR), as characterized by 5% or less blast in the marrow, recovery of neutrophils to at least 1500 µL and RBC and platelet transfusion independent, was 81.8% (9/11). Seven patients receive two MPAC course, two patients did not receive the recommended second M-FAC because of disease progression (one patient) or infection (one patient). Subsequently, M-FAC was given to two patients twice every 4 weeks for maintenance therapy. Seven patients (63.6%) obtained a durable CR and the median response duration was 9.4 months. The most common non-haematological toxicities were infections and elevation of bilirubin and hepatic enzymes. Interestingly, no VOD was noted in this setting, probably because these patients were in CR and the tumour load was low. Severe bleeding occurred in 21% of patients. Such a positive outcome, which compares favorably with that of the few published studies in which a similar approach was used, further supports the use of GO in AML in combined regimens for AML. In a phase II study of patients...
with CD33-positive primary refractory or relapsed AML, the GO, fludarabine, cytarabine and CsA combination regimen resulted in a CR rate of 26% and a CRp rate of 6%. The overall median survival duration was 5.3 months (Tsimberidou et al. 2003). The same GO-containing regimen was used as postremission therapy in patients with AML who were in CR1 after a GO-based induction regimen (Tsimberidou et al. 2008). In our clinic we similarly applied this regimen in CR after standard induction regimens. In this setting MFAC was feasible and well tolerated. Moreover, the CR rates obtained in patients receiving MFAC after standard induction regimens were particularly encouraging. We conclude that combination with other cytotoxic agents may increase GO efficacy in elderly patients with AML. Further trials to optimise the dose and timing of GO administration are warranted.

References


We have sorted Lin CD38 CD44, Lin CD38 CD34, and Lin CD38 CD34 hematopoietic stem cells (HSCs) from 24 CML patients at diagnosis. The gene expression profile was studied using Affymetrix HG-U95Av2 GeneChip array. Molecular caryotyping and quantitative analysis of BCR/ABL transcript demonstrated that about one third of Lin CD38 CD34 were leukemic. CML CD34+ cells showed kinetic quiescence, limited clonogenic capacity and no long-term culture initiating cells (LTC-IC). However, stroma-dependent liquid cultures and cytokines induced CD34 expression on some HSCs, cell cycling, acquisition of clonogenic activity and generation of LTC-IC. In summarization, our results indicate that both BCR/ABL-positive CD34+ cells engrafted in NOD/SCID/beta2null immunodeficient mice similarly to CD34+ cells. Microarray analysis showed a reduced expression of growth factor (GF) receptors in CML as compared to normal HSCs. In addition, when we analyzed the proliferative rate of CML cells with reduced doses of GFs in liquid cultures, we found that Lin CD38 CD34 stem cells showed the same proliferation at 1/10 of the optimal GF dose that cells treated with the full dose. Taken together, these findings suggest that in CML stem cells an autocrine loop for some GFs may be operative. Since Holyoake et al. (Blood, 2002) identified a subset of quiescent Ph CD34+ resistant to imatinib, we studied whether the purified Lin CD38 CD34 may have the same functional activity. As expected, increasing doses of imatinib (0.1 to 10 µM) showed a statistical significant inhibitory effect on the clonogenic activity of CML Lin CD38 CD34 as compared to their normal counterparts. However, we did not find any significant difference when we tested imatinib on Lin CD38 CD34 cells. More importantly, quantitative RT-PCR analysis of BCR/ABL in Lin CD38 CD34 cells did not demonstrate any reduction of the transcript within the range of doses tested whereas we observed a dose-response curve for CD34+ cells. Molecular analysis of the expression of imatinib resistance genes did not show any difference as for ABC transporter family genes and OCT-1 between CD34+ and CD34– cells. In summary, our results indicate that Lin CD38 CD34 cells belong to the stem cell compartment in CML and appear to be poorly responsive to imatinib treatment. Therefore CD34+ cells may represent a potential therapeutic target in leukaemia and further studies are in progress to identify the mechanism of imatinib resistance.

PO-056

A NOVEL SUBSET OF CD34- LEUKEMIC STEM CELLS IN CHRONIC MYELOGENOUS LEUKEMIA (CML) APPEAR TO BE POORLY RESPONSIVE TO IMATINIB TREATMENT IN VITRO

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We have sorted Lin CD38 CD44, Lin CD38 CD34, and Lin CD38 CD34 hematopoietic stem cells (HSCs) from 24 CML patients at diagnosis. The gene expression profile was studied using Affymetrix HG-U95Av2 GeneChip array. Molecular caryotyping and quantitative analysis of BCR/ABL transcript demonstrated that about one third of Lin CD38 CD34 were leukemic. CML CD34+ cells showed kinetic quiescence, limited clonogenic capacity and no long-term culture initiating cells (LTC-IC). However, stroma-dependent liquid cultures and cytokines induced CD34 expression on some HSCs, cell cycling, acquisition of clonogenic activity and generation of LTC-IC. In summarization, our results indicate that both BCR/ABL-positive CD34+ cells engrafted in NOD/SCID/beta2null immunodeficient mice similarly to CD34+ cells. Microarray analysis showed a reduced expression of growth factor (GF) receptors in CML as compared to normal HSCs. In addition, when we analyzed the proliferative rate of CML cells with reduced doses of GFs in liquid cultures, we found that Lin CD38 CD34 stem cells showed the same proliferation at 1/10 of the optimal GF dose that cells treated with the full dose. Taken together, these findings suggest that in CML stem cells an autocrine loop for some GFs may be operative. Since Holyoake et al. (Blood, 2002) identified a subset of quiescent Ph CD34+ resistant to imatinib, we studied whether the purified Lin CD38 CD34 may have the same functional activity. As expected, increasing doses of imatinib (0.1 to 10 µM) showed a statistical significant inhibitory effect on the clonogenic activity of CML Lin CD38 CD34 as compared to their normal counterparts. However, we did not find any significant difference when we tested imatinib on Lin CD38 CD34 cells. More importantly, quantitative RT-PCR analysis of BCR/ABL in Lin CD38 CD34 cells did not demonstrate any reduction of the transcript within the range of doses tested whereas we observed a dose-response curve for CD34+ cells. Molecular analysis of the expression of imatinib resistance genes did not show any difference as for ABC transporter family genes and OCT-1 between CD34+ and CD34– cells. In summarization, our results indicate that Lin CD38 CD34 cells belong to the stem cell compartment in CML and appear to be poorly responsive to imatinib treatment. Therefore CD34+ cells may represent a potential therapeutic target in leukaemia and further studies are in progress to identify the mechanism of imatinib resistance.

PO-057

ADRENOMEDULLIN AND ENDOTHELIN-1 STIMULATE CLONAL GROWTH, BUT NOT IN VITRO EXPANSION OF PBSCs IN PATIENTS AFFECTED BY MYELOMA MULTIPLE

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Introduction. Aderenomedullin (ADM) and Endothelins-1 (ET-1) are two regulatory peptides almost ubiquitously expressed in human tissues where exerting pleiotropic activities on proliferation, differentiation and apoptosis of several cell systems. Recently, some our studies demonstrated for the first time that ADM and ET-1 increase clonal growth of cord blood hematopoietic cells cultured on semisolid media and enhance their in vitro expansion with cytokines. Therefore in this work we have verified if both peptides act in the same way also on peripheral hematopoietic stem cells (PBSCs) mobilized in patients affected by myeloma multiple to better understand their biology. Methods. This check was carried out by means of clonogenic assays and middle-term expansion system in liquid culture medium. Clonogenic assays were performed according to Miller et Lay seeding aliquots of PBSCs in semi-

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solid medium (Methocult GF H4434, StemCells Technologies) with or without ADM (5×10^-8) and ET-1 (5×10^-8) for evaluating the colony forming units (CFU) and the clonal efficiency (CE=CFU/seeded cells number). Middle-term expansion of PBSCs according to Breems’ protocol was used, seeding on 24 wells plate in presence of ADM (2.5×10^-8), ET-1 (2.5×10^-8) and three different cytokine cocktails: A) IL-3, IL-6, SCF, FL3; B) Cocktail A + TPO; and C) Cocktail B + IL-1beta, GM-CSF, G-CSF, EPO. After two weeks of incubation cells were utilized for clonogenic assays in semisolid medium for counting the CFU number. Results. In the clonogenic assays, incubation with ADM and ET-1 evoked a 20.78% and 15.60% rise in the clonal efficiency respectively (CE control: 0.01260.0016 vs CE ADM: 0.01520.0023 p<0.05; CE control: 0.01260.0016 vs CE ET-1: 0.01450.002 p<0.05). In the middle-term expansion, cytokine cocktails A, B and C expanded the hematopoietic progenitors 8.53-fold, 12.55-fold and 8.95-fold with respect to control cultures. The addition of ADM to the above mentioned cocktails gave the following increments of expansion: A=8.86-fold, B=9.53-fold and C=7.41-fold; while the addition of ET-1 produced the following rises: A=9.67-fold, B=7.80-fold and C=9.73-fold. Culture treatment with ADM and ET-1 in the absence of cytokine cocktails increased the CFU number of 1.70-fold and 1.15-fold, respectively. All statistics comparisons between the middle-term cultures with and without ADM or ET-1 resulted not significant (p>0.05). Discussion. Therefore, these experiments showed that ADM and ET-1 are able to stimulate PBSCs growth in single cell suspension. However, in middle-term cultures ADM and ET-1 fail to stimulate PBSCs expansions unlike cord blood where the inductive capacity of both peptides seem to depend on and

**PO-059**

**THE CLEAVED FORM OF SOLUBLE UROKINASE-RECEPTOR STIMULATES MIGRATION AND MOBILIZATION OF MOUSE CD34+ CELLS IN VITRO AND IN VIVO**


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Introduction. Cleaved forms of soluble urokinase-receptor (scuPAR) have been detected in biological fluids of patients affected by various tumors. We recently reported increased scuPAR levels in serum of healthy donors during granulocyte colony-stimulating factor (G-CSF)-induced mobilization of CD34+ hematopoietic stem cells (HSCs), in vitro, c-scuPAR or its derived chemotactic peptide (uPAR84-95) stimulated migration of human CD34+ HSCs by activating receptors for fMet-Leu-Phe (fMLP), a potent leukocyte chemotactrant, and activated CXCR4, the chemokine receptor primarily responsible for HSC reten-

**PO-060**

**AMNIOTIC FLUID CONTAINS MULTIPOTENT MESENCHYMAL STEM CELLS WITH HIGH PROLIFERATIVE POTENTIAL AND SAFETY FEATURES: SOLID PERSPECTIVES FOR CLINICAL APPLICATION**


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Background. Mesenchymal stem cells (MSCs) are multipotent cells considered a promising powerful tool for regenerative medicine and in hematopoietic stem cell transplantation. However, in many clinical conditions cell dose may be the critical factor and the yield resulting from the ex vivo expansion of bone marrow derived MSCs...
may be insufficient. Thus, alternative sources of MSCs need to be explored. In this study, MSCs were successfully isolated from amniotic fluid (AF) and analyzed for chromosomal stability to validate their safety for a potential utilization as cell therapy product.

**Methods.** MSCs were expanded up to the 6th passage starting from second trimester AF using different culture conditions to optimise a large-scale production.

**Findings.** MSCs population was extensively expanded from 21/23 samples of AF. Such cells were characterized by flow cytometry, showing a bright expression of CD73, CD146 and HLA class I antigen, a variable expression of CD44 and CD105 and the lack of haematopoietic and endothelial antigens. We observed a significant correlation between the MSCs yield and the plating density, reaching the highest cell amount at low plating density. Moreover, fetal MSCs revealed to be endowed of significantly higher expansion potential compared to adult BM. AF-MSCs represent a relative homogeneous population of small cells with immunosuppressive properties on T-cell proliferation and extensive proliferative potential. Despite their high proliferative capacity, we did not experienced any karyotypic abnormalities and transformation potential in vitro or any tumor formation in vivo. Interpretation. AF is a rich source of MSCs suitable for banking and universal application to be used when large or repeated infusions of cells are required.

**PO-061**

**PROGNOSTIC VALUE OF POSITRON EMISSION TOMOGRAPHY/COMPUTED TOMOGRAPHY (PET/CT) IN PATIENTS WITH LYMPHOMA TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR RELAPSED/REFRACTORY OR HIGH RISK DISEASE**

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Haematology Unit, 1Department of Medicine, Ca’ Foncello Hospital, Treviso; 1Nuclear Medicine Unit, Castelfranco Veneto Hospital, Italy

PET/CT imaging has increasingly been used for management of malignant diseases including lymphoma. This emerging modality combines the advantages of both PET and CT imaging especially improving specificity. So far no prospective study adressed its prognostic value in the setting of high dose therapy with autologous stem cell transplantation (ASCT). We conducted a prospective study including 6 patients with relapsed Hodgkin Lymphoma, 23 patients with non Hodgkin Lymphoma (19 in relapse, 2 patients in complete remission but at high risk for IPI >3 and 2 patients with Mantle cell Lymphoma treated with HDS regimen: see Table 1 for demographic data).

**Table 1. demographic data.**

<table>
<thead>
<tr>
<th>Age</th>
<th>45 (19-63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>M 18/F 11</td>
</tr>
<tr>
<td>Disease</td>
<td></td>
</tr>
<tr>
<td>Hodgkin Disease</td>
<td>6 Relapse</td>
</tr>
<tr>
<td>Follicular Lymphoma</td>
<td>7 4 relapse</td>
</tr>
<tr>
<td>DLBCL Large B cell Lymphoma</td>
<td>12 3 partial remission or high risk</td>
</tr>
<tr>
<td>T cell Lymphoma</td>
<td>2 1 partial remission</td>
</tr>
<tr>
<td>Mantle cell Lymphoma</td>
<td>2 1 relapse</td>
</tr>
</tbody>
</table>

All the patients presented a positive PET/CT (PET/CT1) before starting treatment. 24 patients underwent PET/CT (PET/CT2) before ASCT and in all of them PET/CT(PET/CT3) was performed after ASCT. After ASCT 6 patients never achieved a CR, of these 2 patients are still alive with disease and 4 died for progressive disease; 20 patients after ASCT were in CR (including 4 patients in CR before ASCT); of these 3 presented a PET/CT positivity 8 months after ASCT followed by clinical and radiological relapse 2 months later. All these patients are still alive with disease. No patients died of treatment-related-mortality. Of note 1 patient presented a PET/CT positivity after the ASCT with a decreasing uptake at subsequent controls and without a clear correlation with any lesion detectable by CT. Median follow up is 18 months (5-57). Overall survival (OS) of whole cohort of patients is 84% and the disease-free-survival (DFS) 66.5%. We did not observed statistically significant differences in OS and DFS on the basis of PET/CT2: 90.9% negative vs 68.1% positive and 65% negative vs 58% positive (see Figure 1).

On the contrary on the basis of PET/CT3 OS is 100% negative vs 56.2% positive, while DFS is 80.8 negative vs 45.4% positive (p<0.01 and p<0.01 respectively by Log Rank test) (see Figure 2). Sensitivity, specificity, predictive positive value and predictive negative value of PET/CT2 and PET/CT3 are reported in Table 2. In this cohort of lymphoma conditions.
patients the role of PET/CT2 in predicting relapse after ASCT is less clear compared to previous published studies, while the role of PET/CT3 seems to be more reliable.

### Table 2. Sensitivity, Specificity, Predictive positive Value, Predictive negative Value of PET/CT2 and PET/CT3.

<table>
<thead>
<tr>
<th>PET/CT2</th>
<th>PET/CT3</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Sensitivity: 0.5556</td>
</tr>
<tr>
<td>Negative</td>
<td>Sensitivity: 0.5556</td>
</tr>
</tbody>
</table>

---

**PO-062**

**PEG-FILGRASTIM SINGLE DOSE VERSUS DAILY FILGRASTIM ADMINISTRATION AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA MALIGNANCIES**

**Tendas A,** 1 Dentamaro T, 1 Cupelli L, 1 Spagnoli A, 1 Bruno A, 1 De Mei, 1, 2 De Angelis V, 1 Datturi T, 1 de Fabritiis P

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**Background.** High dose chemotherapy + radiotherapy followed by autologous stem cell transplantation (aSCT) has a key role in the treatment of haematological malignancies. G-CSF reduces the duration of neutropenia and related infectious complications. Pegfilgrastim (PEG-G-CSF) has been shown to be as safe and effective as daily filgrastim or lenograstim (daily-G-CSF) for mobilization or standard chemotherapy induced neutropenia; limited experiences, however, are available on the use of PEG-G-CSF after autologous transplantation. The aim of this study was to assess the efficacy of single dose PEG-G-CSF (6 mg sc day +4) compared with daily-G-CSF (5 mcg/kg/day sc or iv starting day +1 or +4) after high dose chemotherapy and aSCT. Material and Methods. From October 2004 to October 2006, 17 pts received PEG-G-CSF; median age was 46 (range 16-65); diagnosis was NHL in 13 pts, HD in 2 and MM in 2. Mean dose of infused CD34+ cells was 6.15±2.37×10^6/kg, collected after mobilization with chemotherapy (IEV, IGEV, DHAP, CTX) and daily-G-CSF. Conditioning regimen was high dose melphalan for MM; BEAM, BEAC or Fludarabine for NHL; ThiopetaMel for HD. As historical control, a group of unselected 17 pts, matching PEG-G-CSF group for sex, age, diagnosis, state at transplantation, conditioning regimen and infused CD34+ cells, but treated with daily-G-CSF, was considered. Results. No differences between the 2 groups were seen in median time to WBC >1x10^9/L or PLT >50x10^9/L. A faster (p=0.03) PLT > 20x10^9/L reconstitution was observed in the group of daily-G-CSF (median 15, 8-35) vs PEG-G-CSF group (median 15, 8-35). Similarly a trend (p=0.06) for faster ANC>0.5x10^9/L reconstitution in favour of daily-G-CSF (median 10, 8-15) vs PEG-G-CSF (median 11, 9-16) was seen. Day to reticulocytes >1%, transfusion requirement, infectious episodes, duration of antibacterial or antifungal therapy and time to discharge were similar in the 2 groups. Discussion and Conclusions. This preliminary study showed that both daily and PEG-G-CSF can support haemopoiesis after autograft. A delay in platelets reconstitution was observed in patients treated with PEG-G-CSF, not affecting, however, transfusion requirement and may find explanation in the prolonged pharmacokinetic profile of PEG-G-CSF, caused by constant drug level during aplastic phase and slower clearance after engraftment compared with daily-G-CSF. Larger and randomized studies are needed to validate these preliminary results.

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**PO-063**

**HUMAN PLATELETS LYSE ALLOSTicts AND RAPID AND EFFICIENT EXPANSION OF MESENCHYMAL STEM CELLS FROM SMALL SAMPLES OF BONE MARROW ASPIRATES**

**Capelli C,** 1 Domenghini M, 1 Benzi I, 1 Pedroni O, 1 Bellavita P, 1 Poma R, 1 Rambaldi A, 1 Golay J, 1 Introna M

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**Introduction.** Human mesenchymal stem cells (hMSC) hold promises for the treatment of several human diseases. The most utilised methods to produce MSC include fetal calf serum (FCS) which represents a very serious concern for the safe administration in patients. Methods. We have explored the possibility of substituting the FCS with clinical preparations of platelet lysates (PL). Furthermore we have tested the feasibility of using these culture conditions to obtain clinically useful numbers of MSCs from small bone marrow samples (2-10 mL). Results. Very small volumes of bone marrow samples were processed (from 1 to 5 mL) and seeded in either FCS or PL conditions. 7 different samples were processed. The number of mononuclear cells seeded ranged from 12.8±10⁶ to 24.6±10⁶. After non adherent cells removal, cells were counted at first trypanosynthesis (P1, at day 1±2), and found to be 0.07±0.07x10⁶ in FCS and 0.13±0.08x10⁶ in PL. We then trypsinised the cells at sub-confluency and found 1.6±0.8x10⁶ at P2 (day 1±2), 10.4±4.9x10⁶ at P3 (day 21±5), 59.4±32.4x10⁶ at P4 (day 25±3) and 264.4±200x10⁶ at P5 (day 30±3) for the PL cultures. 5 out of 7 samples have reached P5, so far. By contrast, cells in FBS reached P2 after 25±3 days of culture with a mean 0.1±0.09x10⁶ cells and, so far, only 2/6 cultures reached P5 after 30 days with 0.2±0.2x10⁶ yield. Following a slightly different approach, we chose to trypsinise the cells irrespective of their confluency state at the same days. In this situation, starting from higher numbers of mononuclear cells (varying from 21.2±10⁶ to 212.6±10⁶) from 4 different bone marrow samples (from 5 to 10 mL), we observed at P2 (18 days) 3.5±5.9x10⁶ in FCS versus 1.9±0.8x10⁶ in PL; at P3 (25 days) 3.8±4.8±10⁶ in FCS versus 6.2±5.3±10⁶ in PL, at P4 (36 days) 4.6±4.5±10⁶ in FCS versus 20.7±11.5±10⁶ in PL, while at P5 (day 45) 4.1±5.6±10⁶ in FCS versus 193.8±116±10⁶ in PL. No difference could be found in terms of morphology, differentiation (osteogenic and adipogenic lineages), surface markers (CD45, CD34, CD29, CD90, CD73, CD105, HLA-ABC and HLA-DR) and immunological properties (inhibition of MLR) of cells expanded with PL or FBS. Discussion. We conclude that, irrespective of the timing of trypanosynthesis and passage, PL result in a more rapid and much more significant final yields of MSC.

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**PO-064**

**THE PREDICTIVE VALUE OF FDG-PET/CT AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION**

**Sora F,** 1 Di Nardo P, 2 Chiusolo P, 2 Laurenti L, 1 Di Gaetano AM, 1 Marra R, 1 Leone G, 2 Sica S

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Accurate staging is the basis for the selection of an appropriate therapeutic approach in patients affected by lymphomas. Currently computed tomography (CT) is unable to differentiate between viable tumor, necrosis, or fibrosis in residual masses. Metabolic imaging as FDG-PET could predict tumor viability in residual mass, but require correlation with anatomical imaging. FDG-PET/CT have been recently introduced to improve diagnostic accuracy. FDG-PET/CT was adopted in a series of patients with lymphomas submitted to autologous stem cell transplantation (ASCT) in order to explore its prognostic value. From December 2004 to April 2007 at our Institution 34 consecutive patients (pts), affected by high risk non Hodgkin (NHL-30pts) and Hodgkin’s (HD-4pts) lymphomas, received 2nd line chemotherapy regimen followed by ASCT and at day 100 after ASCT were submitted to FDG-PET/CT. There were 20 males and 14 females, median age was 48 years (range 15-66). NHL subtypes were large B cell in 10 pts, follicular in 8 pts, mantle cell in 5 pts, anaplastic CD30 in 3 pts, peripheral T cell in 3 pts and lymphomasoidoid lymphoma in 1 pt. At diagnosis IV stage was present in 28 pts and 18 pts presented B symptoms. Thirty-one pts received CHOP-like 1st line chemotherapy and 17 received rituximab. Second line chemotherapy was MiCMA in 80 pts, among them 25 pts also received rituximab. Before ASCT disease status was: complete remission (CR) in
14 pts, partial remission (PR) in 15 pts, stable disease (SD) in 4 pts and progressive disease (PD) in 1 pt. Twenty-eight pts are evaluable for PET/CT restaging after ASCT, 6 patients are too early to be evaluated. FDG-PET/CT was negative for residual disease in 18 pts. All but one PET/CT negative pts were alive in CR at a median follow up of 14 months after ASCT, one pt died from CMV/Pneumocystis Carinii pneumonia with no evidence of lymphoma 8 months after ASCT. Ten pts were FDG-PET/CT positive. From December 2004 to April 2007, at our Institution 24 consecutive patients (pts), affected by high risk non Hodgkin (NHL-30pts) and Hodgkin’s (HD-4pts) lymphomas, received 2nd line chemotherapy regimen followed by ASCT and at day 100 after ASCT were submitted to FDG-PET/CT. There were 20 males and 14 females, median age was 48 years (range 15-66). NHL subtypes were large B cell in 10 pts, follicular in 8 pts, mantle cell in 5 pts, anaplastic CD30 in 3 pts, peripheral T cell in 3 pts and lymphoplasmocitoid lymphoma in 1 pt. At diagnosis IV stage was present in 26 pts and 18 pts presented B symptoms. Thirty-one pts received CHOP-like 1st line chemotherapy and 17 received rituximab. Second line chemotherapy was MiCMA in 30 pts, among them 23 pts also received rituximab. Before ASCT disease status was: complete remission (CR) in 14 pts, partial remission (PR) in 15 pts, stable disease (SD) in 4 pts and progressive disease (PD) in 1 pt. Twenty-eight pts are evaluable for PET/CT restaging after ASCT, 6 patients are too early to be evaluated. FDG-PET/CT was negative for residual disease in 18 pts. All but one PET/CT negative pts were alive in CR at a median follow up of 14 months after ASCT, one pt died from CMV/Pneumocystis Carinii pneumonia with no evidence of lymphoma 8 months after ASCT. Ten pts were FDG-PET/CT positive. Four of them showed lymphoma progression and three died from lymphoma at 5, 7, 22 months respectively, 1 pt was rescued by haploidentical SCT. Five pts were in PR at a median of 8 months after ASCT respectively and 1 in CR after 12 months. Two pts received involved field radiotherapy (RT) and both of them achieved FDG-PET/CT negativity and are alive with no evidence of disease at 8 and 18 months respectively. FDG-PET/CT after ASCT has a strong prognostic value both for PFS ($p=0.0044$), it also guided additional RT after ASCT.

Cytokine production in the culture supernatants was quantified by enzyme linked immunosorbent assay (ELISA) when cells achieved confluence after 3 passages, corresponding to the initial phase of their exponential growth. Moreover, CB and BM-MSC were added at different doses ($10^5$, $5 \times 10^5$, $10^5$) to PBL stimulated with Phytohaemagglutinin (PHA) and to Mixed Lymphocyte Cultures (MLC). Lymphocytes proliferation was measured by 5H-Thymidine incorporation. Results. CB and BM-MSC produced substantial amounts of SCF, IL-3, IL-6, TPO but no statistical difference for SCF, IL-3 and TPO. Moreover, CB-MSC cultures produced 10 times more IL-7 than BM-MSC cultures. CD34+ cells alone were not able to grow in culture. CB-MSC and BM-MSC were added at different doses ($10^5$, $5 \times 10^5$, $10^5$) to PBL stimulated with Phytohaemagglutinin (PHA) and to Mixed Lymphocyte Cultures (MLC). Moreover, BM-MSC supported ex-vivo expansion of HPC (2,0±0,3 and 2,5±0,5 NC fold expansion respectively) and their proliferation in CFU assay, whereas, as expected, CD34+ cells alone were not able to grow in culture. CB-MSC did not induce Tcell alloresponse and they inhibited mitogenic lymphocyte proliferation by PHA (48-88%) in a dose dependent manner (Figure 1a). Suppression of Tcell proliferation was observed after the addition of different CB-MSC doses to MLC but, CB-MSC seemed to have a less consistent inhibitory effect (Figure 1b). In conclusion our experiments demonstrated that both CB and BM-MSC have the capacity to secrete some of the most important cytokines involved in expansion of HPC and show an interesting immunomodulatory capacity. Although further studies are needed to investigate more extensively their properties, CB-MSC could represent an alternative source of MSC for improving HSCT outcome.

**PO-065**

**IMMUNOMODULATORY PROPERTIES AND HAEMATOPOIESIS SUPPORTING ACTIVITY OF CORD BLOOD MESENCHYMAL STROMAL CELLS (CB-MSC) VERSUS BONE MARROW MSC (BM-MSC)**

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**Introduction.** Several studies have demonstrated that BM-MSC might play an important role in haematopoietic stem cell transplants (HSCT), reducing GVHD and improving haematopoietic engraftment. The aim of this study is to evaluate the immunomodulatory properties and the haematopoiesis supporting activity of CB-MSC in comparison with those of BM-MSC. Material and Methods: CB-MSC and BM-MSC were isolated by lineage-depletion negative immunoselection (RosetteSep) and density gradient separation (Ficoll) respectively. Both sets of cells were cultured in alpha MEM with 20% FCS and 2 mM L-glutamine. CB derived CD34+ haematopoietic progenitor cells (HPC) were isolated by positive immunoselection using the MidiMACS system. CD34+ cells were seeded on the MSC feeder layers in RPMI medium+10%FCS and co-cultured for 12 days.
HUMAN BONE MARROW MESENCHYMAL STEM CELLS TRANSPLANTATION IN 6-HYDROXYDOPAMINE-LESIONED RODENTS
Bossoniaco P1, Zennarino E1, Armentero M1, Cova L1, Calzarossa C1, Levandis G1, Mellone M1, Blandina F1, Soligo D1, Onida E1, Silani V2, Lambertenghi Deliliers G1, Polli E1
1 Fondazione Materassi, Milano; 2 Dipartimento di Scienze Neurologiche, IRCCS Istituto Auxologico Italiano, Università di Milano, Milano; 3 IRCCS Fondazione Istituto Neurologico C. Mondino, Laboratorio di Neurochimica Funzionale, Pavia; 4 Ematologia, Centro Trapianti di Midollo, Ospedale Maggiore IRCCS, Università di Milano, Milano, Italy

Introduction. Parkinson’s disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons. The aim of this work was to investigate the mesenchymal stem cells (MSC) capacities to migrate, differentiate and to support neurons survival when transplant- ed in the 6-hydroxydopamine (6-OHDA) animal model of PD. Methods. For in vitro studies, human commercial MSCs were expanded, charac- terized for the expression of lineage specific markers and their differen- tiation capacities toward adipogenic and osteoblastic phenotypes. Morphological analysis, apoptosis (TUNEL analysis) and viability (Ki67 stain- ing) were also tested. The expression of neural specific markers in basal conditions was evaluated by RT-PCR, immunocytochemistry and West- ern Blot. Neurotrophic factors production was quantified by ELISA. In order to assess the effect of 6-OHDA, MSCs were exposed to different concentrations of the toxin. The protective effect of MSCs was also test- ed by co-culture with neural progenitors. For in vivo studies, 32,000 or 180,000 Hoechst-labeled human cells were transplanted in the ipsilater- al striatum of Sprague Dawley rats 5 days after injection of 6-OHDA, under cyclosporine A immuno suppressive treatment. To evaluate the in vivo effect of transplanted MSCs and animal behaviour to lesion, apo- morphine-induced rotational test was performed. Finally, 28 days after the lesion was performed, animals were sacrificed and brains sections were analyzed by immunocytochemistry for proliferation of transplant- ed and endogenous cells, astroglial and microglial response and dopaminergic terminals damage. Results. In vitro, MSCs expressed typi- cal mesenchymal stem cells markers, were able to differentiate into adipocytes and osteoblasts and had an active and healthy metabolic state. They display low expression of neuro-glial antigens but pr

THE ROLE OF RADIOMUNOTHERAPY (RIT) AND HEMOPOETIC STEM CELL TRANSPLANTATION (HST): ANALYSIS OF TEN CASES WITH MALIGNANT NON HODGKIN LYMPHOIMA
Rana A, Mele A, De Francesco R, Del Casale C, Messa AR, Greco G, Sibilla S, Frusciante V1, Varraso A1, Dicembrino F1, Tabacco I, Caputo M1, Musarò A1, Ostuni A1, Pavone V
Department of Haematology, Department of Transfusion Medicine, Hospital Card. G. Panico, Tricase (Le); 1Department of Nuclear Medicine, Hospital Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy

Radioimmunotherapy (RIT) with 90Y-Zevalin® produces a high response rate in patients with recurring non Hodgkin Lymphoma (NHL). Recently, 90Y-Zevalin® has been studied as part of a conditioning regi- men prior to autologous and reduced-intensity allogeneic stem cell trans- plantation with interesting results. We evaluated the feasibility and the clinical results of the addition of 90Y-Zevalin® at standard dose (0.4 mCi/Kilograms) to a BEAM high dose regimen in 10 patients with advanced stage NHL who failed to achieve complete remission (CR) after first line chemotherapy. Methods. The treatment plan is shown in Figure 1. PBSCs were collected after mobilization with DHAP and G- CSF. Patients’ characteristics are shown in Table 1. Results. The median CD34+ cells infused was 5.04×10^6/Kilograms (range 3.22-21.6). All patients engrafted. The median number of red blood cell and platelet transfusion were 4 (1-7) and 6 (1-5), respectively. The median time to platelet counts higher than 20×10^9/L were 16 days (range 9-28 days). The median time to an absolute neutrophil count greater than 0.5×10^9/L were 10 days (range 8-14). The average numbers of CD3+, CD4+, CD8+ and CD56+ cells on day + 90 are summarized in Table 2. Mucosites occurred in all patients (grade III in 6 cases and IV in 1 case). Febrile neutropenia occurred in 80% of cases. Five pneumonitis and 5 blood stream infections, mainly by Gram 1, were documented. One patient developed an atrial fibrillation. The 90-day response demonstrated a complete response (CR) rate of 60% (6 cases) and a partial response (PR) of 40% (4 cases). Three PR patients progressed with a median follow-up of 150 days (60-300). Two of these died for the progression disease on day 270 and 90, one is alive in PR after radiotherapy. Two CR patients died on day 30 and 60 for septic shock and viral encephalitis (median age 71 years). With a median follow-up of 165 days (30-390) 5 patients are in CR (one developed a myelodisplastic syndrome on day 200), 1 in RP and 4 died. Conclusions. In summary, the use of RIT plus transplant induces 50% of CR with sustained engraftment, an acceptable extra-haemato- logical toxicity and a rapid immunological recovery in patients who failed to achieve CR after first line chemotherapy. The power of this pro- gram needs to be assessed in a larger series of patients and in a multi- center setting.

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Median age, y (range)</th>
<th>53 (25-70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histology, no. (%)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>Follicular</td>
<td>7 (70)</td>
</tr>
<tr>
<td>Aggressive</td>
<td>9 (80)</td>
</tr>
<tr>
<td>III-IV stage at diagno-</td>
<td>2 (2-4)</td>
</tr>
<tr>
<td>sis, no. (%)</td>
<td></td>
</tr>
<tr>
<td>II-III</td>
<td>8 (80)</td>
</tr>
<tr>
<td>Bone marrow involveme-</td>
<td>6 (60)</td>
</tr>
<tr>
<td>nt at diagnosis, no. (%)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Prior rituximab, no. (%)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Status at transplant</td>
<td>13 (8-72)</td>
</tr>
<tr>
<td>Progression</td>
<td>13 (8-72)</td>
</tr>
<tr>
<td>PR8 (80)</td>
<td>13 (8-72)</td>
</tr>
<tr>
<td>Median time to HST, months (range)</td>
<td>13 (8-72)</td>
</tr>
</tbody>
</table>

Table 2. Recovery of CD3+, CD4+, CD8+ and CD56+ cells.

| CD3+ Cells | 1035 (160-2915) |
| CD4+ Cells | 310 (60-1024)  |
| CD8+ Cells | 645 (90-2730)  |
| CD56+ Cells | 65 (20-148)  |
PO-068
USE OF KGF (PALIFERMIN) BUT NOT OF G-CSF IS ASSOCIATED TO A REDUCED RATE OF INFECTIONS AFTER HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS PBSCT TRANSPLANTATION
Divisione di Ematologia e Unità di Trapianto di Midollo, Ospedale Ferrarotto-Catania, Italy

Introduction. Reduction of infections after PBSCT autologous transplantation is an important clinical goal. Use of G-CSF has been shown to shorten neutropenia without improving infectious risk. KGF (Palifermin) has been shown to reduce febrile neutropenia when used during TBI-containing regimens, however, efficacy of Palifermin has not been determined when used in association with non TBI containing regimens.

Methods. We have studied factors associated to infections in a group of 156 patients, all received a non TBI-containing eradicating regimen and were treated with either G-CSF and/or Palifermin. G-CSF was used according to two randomised studies run in our institution while PALIFERMIN was used sequentially in a cohort of 29 patients. Mean age was 49 years, underlying diagnosis was MM (77 pts), Lymphomas (66 pts), other (13 pts), 52% of patients were in advanced phase of disease, conditioning regimens were: L-PAM (n.77), BEAM (n.60), other (n.19), dose of infused CD34+ was 5.5×10^6/Kg. Anti-infectious prophylaxis was standardised and comprised in all patients systemic antibiotic, intestinal decontamination, acyclovir, fluconazole. Results. Neutrophil engraftment was reached in a mean of 12 days, Febrile neutropenia was diagnosed in 45% of patients, Gram-negative Bacteremia in 4.5%, Pneumonia in 5.1%, CVC-associated Bacteremia in 16% of patients. TRM at 1 year was 0%. Results of association of various factors to Severe Infections, defined as diagnosis of FUO or GRAM- BACTERIAEMIA or PNEUMONIA in univariate and in multivariate logistic regression are report ed in the following Table 1.

Table 1.

<table>
<thead>
<tr>
<th>FACTORS EVALUATED FOR SEVERE INFECTIONS: FUO or GRAM- BACTERIAEMIA or PNEUMONIA or GRAM + BACTERIN</th>
<th>UNIVARIATE LOGISTIC REGRESSION</th>
<th>MULTIVARIATE LOGISTIC REGRESSION</th>
<th>ODDS RATIO (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DH HD-PAM CONDITIONING</td>
<td>p=0.004</td>
<td>p=0.09</td>
<td></td>
</tr>
<tr>
<td>USE OF PALIFERMIN</td>
<td>p=0.002</td>
<td>p=0.008</td>
<td>0.267 (1.00-0.716)</td>
</tr>
<tr>
<td>DISEASE IN ADVANCED PHASE</td>
<td>p=0.002</td>
<td>p=0.23</td>
<td></td>
</tr>
<tr>
<td>SEVERE MUCOSITIS</td>
<td>p=0.025</td>
<td>p=0.03</td>
<td>2.193 (1.06-4.401)</td>
</tr>
<tr>
<td>AGE</td>
<td>p=0.10</td>
<td>p=0.34</td>
<td></td>
</tr>
<tr>
<td>USE OF G-CSF</td>
<td>p=0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEUTROPILS AT ADMISSION</td>
<td>p=0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIME TO PHN+0.5×10^9/L</td>
<td>p=0.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion. In conclusion use of PALIFERMIN is the only modifiable factor that reduces significantly the risk of Severe Infections after high dose non TBI-containing regimens and PBSCT transplantation.

PO-069
EARLY AND LATE COMPLICATIONS IN 286 ADULT AUTOLOGOUS STEM CELL TRANSPLANT RECIPIENTS
Scollo C, Terruzzi E, Rossini E, Piotelli P, Parma M, Verga L, Pogliani EM
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Introduction. High-dose therapy supported by autologous stem cell transplantation (ASCT) has become a widely applied therapy in many haematological malignancies during two decades. ASCT may be curative and even more commonly leads to prolonged survival. The early treatment-related mortality (<100 d) has reduced (2-3%). Also late (>100 d) non-relapse mortality (NRM) can occur. Methods. We have analysed a cohort of 221 patients (pts) who received ASCT (286 transplant) from 1994 to 2007 in our department. The median age was 49. We treated: lymphoma (108), multiple myeloma (82), acute leukaemia (34), chronic lymphocytic leukemia (2) and myelodysplastic syndrome (2). At the time of transplant 16 pts were in complete remission, 82 in partial remission and 88 in persistent/progression malignancies. The most common treatment before ASCT included: Alkeran (200 mg/m² or 140 mg/m²): 55%, BEAM 30%, Busulfan-Cyclophosphamide 4%, others 11%. Results. The median dose of CD34+ infused was 5.21×10^6/kg. The median time to achieve an absolute neutrophil count >0.5×10^9/L was 12 days. The median time to achieve a platelet count >20×10^11/L was 19. 192 patients (67%) developed oral mucositis:125 (64%) grade 1-2, 69 (36%) grade 3-4. High dose chemotherapy with BEAM was more toxic on the oral mucose than high dose of Alkeran alone. 43% of patients needed total parenteral nutrition support. 209 pts (73%) developed sepsis: FUO 61%, Gram + 20%, Gram – 12%, miscellaneous 3%, fungi 2%, viral 1%, Pneumocistis Jiroveci (Cariini) 1%. Complications including cyclophosphamide were responsible of the most number of sepsis. 45 pts presented a positive antigenemia of Cytomegalovirus, all of them were sieropositive for CMV before ASCT; only one case of primary infection was seen. The late complications were: cardiac (30%), hepatic (16%), renal (3%), gastrointestinal (12%), SNC (8%), others (MGUS, cataract, aseptic necrosis of bones, diabetes 31%). The most important cardiac complications were: atrial fibrillation, sinus tachycardia, arrhythmia, peri-carditis. 61 pts (32%) died after ASCT.62% of them died for malignancy relapse. 25% of pts died for infections. 2 pts died for a secondary acute myeloid leukaemia. Fatal cardiovascular complications were observed in 4 pts (acute myocardial infarction or cardiomiopathy). Discussion. ASCT is a safe procedure. In our institution infections represent the most important early complications, otherwise they aren’t responsible of late mortality.

PO-070
HPSC: POLYMORPHISM FREQUENCY IN SARDINIAN RECIPIENT/DONOR AND UTILITY FOR CHIMERISM STUDIES
Unità Operativa di Ematologia e Centro Trapianti Midollo Osseo, Ospedale Oncologico A.Businco Asl 8 Cagliari, Italy

Introduction. Micro satellites are tandem repetition of sequences that randomly occur in eukaryotic genomes. They are easy to be characterized and may display considerable polymorphism due to variation in number of repeats VNTR. This polymorphisms are sufficiently stable to use in genetic analysis and are ideal markers to identify susceptibility loci involved in common genetic disease. Parents are often heterozygous in microsatellite locus and segregations of alleles can be observed unambiguously in the progenies. Several micro satellites have four or more alleles and have Polymorphic index level (PIC) > 0.7. This high polymorphic microsatellites are fully informative in the 50% of families. Frequency of micro satellites are different in Caucasian people, we use shorter sequences than variable number tandem repeats(VNTR) and short tandem repeats (STR) in Sardinian people as isolated population is ideal for polymorphism studies. Objective. To identify frequencies of microsatellites in Sardinian population (recipient and donor) with PCR typing procedure in order to verify number of polymorphic genes in chimerism studies. Design. All polymorphisms have been evaluated with Polymerase chain reaction, this procedure was evaluated on 2% agarose gel electrophoresis (VNTR) and 6% acrilamide gel electrophoresis (STR). Result. From 2000 to may 2007, two hundred cases were evaluated (98 recipient and 102 donors). In all cases 10 different polymorphisms gene were tested (DYS385, MCT118, PAH, APO B1, APOC1, YNZ-22, IGF1, CAR, 33.6, SE33). Six were STR and 4 VNTR. In Sardinian people 34% were informative for MCT118 gene, 33% PAH, 10% APOB1, 5% YNZ-22, 4% SE33.5, 2% 33.30, 1% APOC1. MCT118 and PAH have a greater frequency with low PIC. Conclusions: Above 98 recipients, 27% (15%) were informative for one polymorphic gene, while 18 (5%) were informative for two polymorphic gene and only 21% were informative for three polymorphic gene. Just 8 cases (4%) weren’t informative on VNTR and STR, then Y chromosome linked short tandem repeats marker was used. In harvest of donor peripheral stem cell we performed a first study panel with a greater informative Sardinian population polymorphism gene. MCT118 on D1S80 locus in 1p36p35 and PAH on 12q2 were informative polymorphisms with greater frequency. Data can be used in diagnostic studies to perform a specific polymorphism panel in informative chimerism analysis.

haematologica/the hematology journal | 2007; 92(s3) | 83
PO-071
 Feasibility of autologous transplantation after THADD regimen in elderly multiple myeloma patients
 Offidani M,1 Ruggieri M,2 Folloni C,1 Corvatta L,2 Scortechini L,1 Montanari M,1 Piersanti M-N,1 Catanini M,1 Burattini M,1 Olivieri A,1 Marconi M,1 Mele A,1 Visani G,1 Leoni P,1 Galieni P,1
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Autologous hematopoietic stem cell transplantation (HSCT) seems to be a feasible and effective in selected MM patients aged over 65 years although it has not been yet established whether new drugs have to be incorporated into induction therapy prior HSCT. Here we assessed the feasibility and toxicity of HSCT performed in patients aged >65 years who had received as front-line therapy ThaDD regimen (Blood, 2006). We studied 20 newly diagnosed MM patients treated with ThaDD regimen followed by allogeneic (2 patients) or autologous (18 patients) HSCT. Median age of 18 patients receiving autotransplant was 67 years (range 65-72). ISS was II-III in 10 patients (55%) and 4 patients (22%) had unfavourable cytogenetics. Overall, 14 patients (78%) underwent single and 4 (22%) double autologous HSCT. Conditioning regimen consisted of high-dose melphalan (200 mg/m²) in 10 cases (55%) and intermediate dose (100 mg/m²) in 8 patients (45%). Median CD34+ cells collected after high dose cyclophosphamide and hematopoietic growth factors was 5.7×10⁶/kg (range 2.8-11.3) whereas median CD34+ cells reinfused resulted 4.1×10⁶/kg (1.2-5.6). Median time to neutrophils > 500/µL was 11.5 days and 13.5 days after first and second transplant, respectively, whereas median time to platelets > 20000/µL was 14 days and 18 days after first and second transplants, respectively. Only 3 patients (17%) developed neutropenic fever of unknown cause. Non hematological toxicity consisted of grade 2 mucositis in 2 patients (11%) and grade 2 diarrhea in another one. No transplant related death was observed. Five patients (25%) achieved a CR, 1 (5.5%) a nCR, 9 (50%) a VGPR and 3 (16.5%) a PR. Remarkably, after autologous HSCT 46% of patients had an improved response if compared with those achieved following ThaDD regimen to first line treatment. VGPR was obtained in patients with CR1 > 12 months as opposed to 13% in those with CR1 duration < 12 months. Alternative approaches, based on combination of salvage therapy with investigational drugs should be offered to high risk patients.

PO-073
 Patients with relapsed Hodgkin’s disease and diffuse large B cell non-Hodgkin’s lymphoma achieving complete response only after autologous stem cell transplantation need further therapeutic intervention
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Introduction. Relapse is the primary cause of failure in patients with Hodgkin’s disease (HD) and diffuse large B cell lymphoma (DLBCL). Standard dose salvage chemotherapy is effective in a minority of patients with recurrent or refractory disease, while autologous stem cell transplantation (ASCT) results in long-term disease control in approximately 35 to 40% of patients. However, while ASCT cures nearly 50% of patients with HD and DLBCL in chemoresistant relapse, it is ineffectual in most case of chemoresistant relapse. Notwithstanding, a minority of patients with relapsed refractory disease can achieve complete response (CR) after ASCT. Few studies have specifically explored the clinical outcome in terms of relapse free survival (RFS) and overall survival (OS) of patients in chemoresistant relapse who achieve CR only after ASCT. Methods. The aim of this study was of investigating the quality of CR achieved in 70 relapsed patients, 20 with HD and 50 with DLBCL, autografted with active disease after salvage therapy and achieving CR only after ASCT. 45 received ASCT after a partial response (PR) and 25 in refractory relapse (RR). The median age of the whole patient population was 54 years (40 years for patients with HD and 57 years for those with DLBCL). Salvage treatment consisted of ifosfamide, epirubicin and etoposide (IEV) in 33 patients, Rituximab + IEV in 17 patients (all with DBLCL) and Cisplatinum, Ara-C and dexamethasone (DHAP) in 10 patients. All PR and 10 RR patients were conditioned with BEAM, while 15 RR cases received the BCV regimen. There were no significant differences between PR and RR patients as age, serum LDH and duration of CR1 are concerned. Results. Relapse rate after ASCT was 42% in PR group as opposed to 90% in RR group (p<0.003). Median relapse free survival from ASCT was 7 months for RR patients as opposed to 32 months for PR patients (p=0.003); median overall survival from ASCT was 10 months for RR subset as opposed to not reached for RR subgroup (p<0.001). According to initial diagnosis, there were no significant differences between patients with HD and DLBCL within each subgroup (i.e. PR and RR). Discussion. CR achieved after ASCT in either HD or DLBCL patients who are refractory to salvage therapy does not result in long-term disease control. Alternative preparative regimens, allogeneic SCT, monoclonal antibodies or radioimmunoconjugates in the post-ASCT phase should be considered for RR patients despite CR achievement.

PO-072
 Prognostic factors and therapeutic results for patients with acute myeloid leukemia relapsed after autologous stem cell transplantation
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Introduction. Autologous stem cell transplantation (ASCT) represents a standard post-remission treatment for acute myeloid leukemia (AML). The increasing use of peripheral blood stem cells has reduced morbidity and mortality of the procedure, without affecting the relapse rate, which still accounts for 30-50% of patients. In this study we focused on prognostic factors and clinical outcome of patients with AML who relapsed after ASCT. Methods. Data were collected from a series of 56 AML patients relapsed after ASCT performed in patients aged >65 years. The median interval from diagnosis to ASCT was 4 months (3-6). Results. Aggressive salvage chemotherapy (SC) was given to 42 patients out of 56 (75%). Reasons of exclusion were: fulminant relapse with early death (2 cases), age over 65 years (12 cases). CR2 was achieved in 13/42 patients (31%). The only factor significantly related to CR2 achievement was duration of CR1; more in detail, CR2 was obtained in 53% of patients with CR1 >12 months as opposed to 13% of patients with CR1 <12 months (p<0.007). On the contrary, other parameters such as cytogenetics, FLT3 mutations, WBC count, number of courses to CR1 achievement had negligible impact on CR2 achievement. Of note, no patients aged over 60 receiving SC (n=9) achieved CR2. The median duration of CR2 was 10 months (2-55). Median survival (OS) from relapse for the whole patients population was 11 months; according to treatment, OS was 6 months for patients receiving palliation as opposed to 12 months for those given SC. However, by considering only patients with CR1 duration < 12 months the advantage of aggressive salvage was of only 3 months (6 vs. 9, p=0.03). CR1 duration was also the only parameter significantly related to survival from relapse (24 months for patients with CR1 >12 months vs. 7 months for the opposite group, p<0.0001). Again, cytogenetics, FLT3 mutations, WBC count and number of courses to CR1 had no prognostic relevance on survival either in univariate or multivariate analysis. Discussion. Therapeutic results in patients with AML relapsed after ASCT are poor, mainly when CR1 duration is shorter than 12 months. Alternative approaches, based on combination of salvage therapy with investigational drugs should be offered to high risk patients.

POSTERS
PO-074
DURABLE REMISSION INDUCED BY HIGH DOSE DONOR LYMPHOCYTE INFUSIONS (DLI) IN TRANSFORMED MYELODYSPLASIA FAILING TWO ALLOGENEIC BMT AND CONVENTIONAL DOSE DLI

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The efficacy of DLI for myelodysplasia (MDS) relapsing after allogeneic bone marrow transplantation (BMT) has been questioned. We report about a 55-year-old patient presenting with a cytopenic 5q-MDS in 1997. Methods. After failure on CSA, he received a first allogeneic BMT from his sister on 3/7/1998, after conditioning with thiopeta 10 mg/Kg and cytoxan (Cy) 100 mg/Kg. He achieved full donor chimerism with little graft versus host disease (GVHD) and relapsed one year later. He received a second allogeneic BMT from the same donor on 12/6/2000, prepared with TBI 100 rads + thiotepa 10 mg/Kg + Cy 100 mg/Kg, with return to full donor chimerism. The same donor on 13/8/1999, prepared with TBI 100 rads + thiotepa 10 mg/Kg, was used for MDS. Results. Three months after a bone marrow aspiration showed no evidence of blasts. Blood counts recovered, but the patient was still transfusion dependent. We managed to raise blood cell counts by 2 weeks of G-CSF treatment. In 2001, he developed skin extensive erythema and later relapse. Therefore, this control was carried out evaluating the clonal growth potential of disease clonogenic cells, which are responsible of bone marrow reconstitution. Instead, treatments (2h and 24h) with GO on CD34+38– isotype in vitro purging procedures. However, data from clonogenic assays with cryopreserved cells suggest to do not use these last because thawing seems to increase GO cytotoxicity. In conclusion, these data permit the start up of new experimental phases to study the use of GO in ex vivo purging of aphaeretic cells of patients affected by AML.

PO-075
EFFECTS OF GENTUZUMAB-OZOGAMICIN ON HEMATOPOIETIC CELLS OF PERIPHERAL AND CORD BLOOD

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In patients affected by Acute Myeloid Leukemia (AML) without matched HLA donors and in aged patients an alternative therapeutic option to allogeneic transplant could be autologous transplant after ex vivo purging of minimal residual disease with Gentuzumab-Ozogamicin (GO). GO, recently introduced in clinical practise, is an anti-CD33 monoclonal humanized antibody which is joined to a calicheamicin derivat. This drug represents the first example of anti-body-target therapy for AML where 90% of blast cells over expressed this antigen. However, its further use for purging is subordinated to the absence of cytotoxicity on normal pluri/multi potent hematopoietic cells, which are responsible of bone marrow reconstitution. Therefore, this control was carried out evaluating the clonal growth of hematopoietic stem cells (HSCs) and CD34+38– subset on peripheral and cord blood. Methods. For this experimentation, aliquots of peripheral and cord blood HSCs, were obtained from Boyum techniques (HSCs) and after (HCSs) cryopreservation, while CD34+38– cells were isolated by negative immunomagnetic selection from fresh samples. Cells were then treated with GO (5 micrograms/mL) for 2 and 24 hours. At the end of incubations clonogenic assays were performed according to Miller et Lay for evaluating the colony forming unit (CFU) number. Clonal growth was determined as clonal efficiency (CE= CFU/seeded cells number). Results. In the treatment for 2h of the peripheral HSCs, the median CE’s of control cultures were 0.01580 and 0.05541 for fresh and cryopreserved cells respectively, in the treated cultures they decrease to 0.01284 and 0.05530 respectively. All statistics comparisons resulted not significant (p>0.05). Analogous decrease was observed with cord blood HSCs (HSCsA control CE= 0.01073; HSCsA control CE= 0.01039; HSCsB treated CE= 0.00724; HSCsA treated CE=0.006636). Statistic comparisons were significant (p<0.05) only for HSCsA. The treatment of 24h with GO significantly reduced the CE in all experimental groups. Instead, treatments (2h and 24h) with GO on CD34+38– isolated from cord blood HSC reduce, but not significantly, the median CE values (HSCsB control CE= 0.02651; HSCsA treated CE= 0.01151). Discussion. The most considerable result of these experiments is the demonstration of low toxicity exercised by GO on CD34+38– cell subset. The results of clonogenic assays with peripheral HSCs seems to confirm this observation. In Literature, was reported how CD34+38– subset is represented by an heterogeneous cell populations with pluri/multi potent functionalities and able to long term bone marrow reconstitution. Therefore this experiment with GO on CD34+38– seems to confirm the hypothesis of its use in ex vivo purging procedures. However, data from clonogenic assays with cryopreserved cells suggest to do not use these last because thawing seems to increase GO cytotoxicity. In conclusion, these data permit the start up of new experimental phases to study the use of GO in ex vivo purging of aphaeretic cells of patients affected by AML.

PO-076
SINGLE VERSUS TANDEM HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN HODGKIN’S LYMPHOMA PATIENTS NOT ACHIEVING COMPLETE RESPONSE OR RELAPSING AFTER INDUCTION CHEMOTHERAPY

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Introduction. We compared the results of single and tandem high-dose chemotherapy (s-HDC, t-HDC) with autologous stem-cell rescue in 68 patients with refractory or relapsed Hodgkin’s lymphoma (HL) after front-line chemotherapy. Methods. All patients received ICE as salvage chemotherapy. Twenty four (35%) patients underwent s-HDC and 44 (65%) t-HDC. At the time of transplantation in s-HDC group, 14 (58%) patients were in CR; 8 (33%) PR, 2 (8%) in PD. In t-HDC group, 21 (48%) patients were in CR, 20 (45%) in PR and 3 (7%) in PD. S-HDC consisted in TM (Thiothepa 600 mg/m² day -1, 160 mg/m² day – 2) followed by BEAM in 2 patients (8%) and Melphalan (MEL 200 mg/m²) in 2 patients (8%); t-HDC first course, consisted in MEL and the second course in BEAM. Results. After s-HDC 21 (87.5%) patients were in CR, 1 (4%) in PR and 2 (8%) patients did not perform HDC for toxicity. At last follow-up, 15 out of 24 patients (62.5%) were alive and in CR; 9 have died: 5 of PD, 1 of secondary leukaemia, 1 of heart arrest and 2 of infections. With a median follow-up of 60 months, survival at five years was 75% and 60%, respectively. The transplant-related mortality (TRM) was 16%. After t-HDC, 36 (82%) patients were in CR, 1 (2%) in PR and 4 (9%) in PD and 2 (4%) did not perform second HDC for PD. At the last follow-up, 32 out of 44 patients (73%) were alive, 30 in CR, 1 in PR after reduced-intensity allotransplant (RIC) and 1 in PD. Eight patients died because of PD (5 progressed after second HDC, 2 did not perform second HDC for PD and 1 progressed after RIC), one patient died of pancreatic neoplasia, two of toxicity related to second HDC and one of aGVHD after RIC. With a median follow-up of 30 months, the 3-year OS and FFP were 76% and 80%, respectively. No statistical difference was achieved comparing OS in the two groups. By contrast there was a trend towards a statistical significant difference in FFP in favour of the t-HDCT group (p=0.06) (Figure 1). TRM was 16%. No statistical differences were seen in hematological toxicity. Severe mucositis was observed in 58% of s-HDC group, 45% in the first and second HDC (p=0.02 and p=0.01). Discussion. T-
HDC seems to improve clinical results compared to a standard approach with s-HDC. Long-term follow-up, a larger cohort of patients and a randomized study will be needed to confirm the overall efficacy of this strategy and to evaluate long-term toxicity.

**Figure 1a. FFP, Freedom From Progression; s-HDC, single – High dose chemotherapy; t-HDC, Tandem – High dose chemotherapy.**

**Figure 1b. OS, Overall Survival.**

**PO-077**

**PURGING IN VIVO AND AUTO-TRANSPLANTATION IN PATIENTS WITH POOR PROGNOSIS LYMPHOPROLIFERATIVE DISEASES**


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Autologous stem cell transplantation is an efficacy therapy for lymphoproliferative disease. However a concern with the procedure is the potential of malignant cells to reinfuse with stem-cell graft. In the past five year, investigators have used rituximab to purge malignant cells in vivo without any manipulation in vitro. From April 2003 to May 2007 we have treated with Autologous stem cell transplantation, purged in vivo with monoclonal antibodies, 21 patients (5 F; 16 M median age: 57 years) with lymphoproliferative diseases to poor prognosis (2 Burkitt lymphoma; 2 Burkitt like lymphoma; 4 mantle cells; 5 CLL; 1 NHL peripheral T cells) and 5 in resistant disease (2 CLL; 2 large cells and 1 NHL peripheral T cells). All patients have harvest (median CD34:4x10^6/Kg) and median minimal residual disease in the marrow has been < to 2%. All the patients have been conditioned with BEAM and the graft are documented in 19/21 patients (2 patients are dead to the day +4 and +10 for gastric haemorrhage and septic shock respectively) with neutrophils>1000 in median to day +14 (range 10-19 days). After transplantation 17/19 patients were in CR, a day +60 the MMR in bone marrow was <0.5% (range 0-0.3%). With a median follow-up of 12 months after transplantation (range 2-50) 14/17 (82%) patients are in CR (3 patients have relapsed: 1 burkitt lymphoma (is relapsed extra-nodular at months +3 and died for disease a months + 5 after transplantation); 1 mantle cells and 1 NHL-peripheral T-cells at +10 and +15 months respectively. Two patients (1 large cells and 1 CLL) are died at months +5 and +7 for CMV reactivation and interstitial pneumonia respectively. The DFS and OS projected at 50 months are of the 65% and 57% respectively (Figure 1).

In conclusion the purging in vivo with antibodies monoclonal, effected during the harvest that immediately after the infusion of the stem-cells, allows to get besides a graft with least residual disease in this cohort (patients with poor prognosis) and the preliminary results they seem excellent. The principal problem in these patients have been primarily the infectious and gastro-intestinal complications, these has been correlated to patients over treated and in disease. These data suggest treating in first line, with transplantation of stem-cells purged in vivo with monoclonal antibodies to eradicate the MRD, patients to poor prognosis or with chronic lymphoproliferative disease.

**PO-078**

**EVALUATION OF PRIMITIVE BFU-E IN HEMATOPOIETIC RECONSTITUTION AFTER AUTOLOGOUS TRANSPLANTATION OF PERIPHERAL BLOOD STEM CELLS**


**Area Trapianto-Clinica Ematologica Fondazione IRCCS Policlinico San Matteo Pavia; Clinica Ematologica Fondazione IRCCS Policlinico San Matteo Pavia; Servizio di Immunonematologia e Trasfusionale, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy**

In semisolid cultures, primitive BFU-E (PB FU-E) are well recognizable and are defined as those progenitors that give rise to more than 16 clusters of hemoglobinized erythroblasts (i.e. colonies consisting of more than 500 cells). They are known as indifferenitated erythroid colonies, close to CFU-MIX colonies, and they generally represent a minority of the BFU-E scored. Sometimes, in the analysis of cultures from collections of peripheral hemopoietic cells (PHSC) mobilized with G-CSF in view of autologous transplantation (BMT), PB FU-E outnumber than mature BFU-E. We have evaluated the meaning of these colonies for the hematopoietic reconstitution (i.e. time to reach the take) in course of PHSC BMT. We have scored the presence of PB FU-E in 75 consecutive cases submitted to leukapheresis for HSC collections (22 Non Hodgkin lymphoma -NHL, 13 Hodgkin Lymphoma –HL, 29 Multiple Myeloma -MM,
PO-079

EVALUATION OF THE MOBILIZING CAPACITY OF G-CSF AND PEG-G-CSF IN MULTIPLE MYELOMA PATIENTS

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We treated 21 multiple myeloma patients with DCEP regimen as mobilizing chemotherapy followed either by G-CSF (lenograstim) or by Peg-G-CSF (pegylate filgrastim). All 21 patients did subsequently undergo autologous transplantation with the hemopoietic peripheral stem cells (HSC) collected. We analysed the time to mobilization, the number of peripheral blood stem cells (PBSC) and CD34+ cells collected, and the days to PMN ≥500x10⁹/L after transplantation. The aim of the study was to determine whether any of these parameters were influenced by the administration of either G-CSF or Peg-G-CSF. The results are reported in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>G-CSF</th>
<th>Peg G-CSF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>16</td>
<td>5</td>
<td>0.9</td>
</tr>
<tr>
<td>Days for mobilization (range)</td>
<td>11-14</td>
<td>10-14</td>
<td>0.9</td>
</tr>
<tr>
<td>Mononuclear Cell x10⁸</td>
<td>273.1±140</td>
<td>423.6±221</td>
<td>0.14</td>
</tr>
<tr>
<td>CD 34+ x10⁶</td>
<td>594.8±370</td>
<td>973.3±629</td>
<td>0.15</td>
</tr>
<tr>
<td>CFU-GM x10⁶</td>
<td>7851.3±134</td>
<td>7105.6±209</td>
<td>0.8</td>
</tr>
<tr>
<td>BFU-E x10⁶</td>
<td>12858±7871</td>
<td>13434±8438</td>
<td>0.8</td>
</tr>
<tr>
<td>Time to Take after BM (days)</td>
<td>13±1.7</td>
<td>14±0.7</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Our data does not show significant difference in all the observed parameters, indicating that the administration of G-CSF or Peg-G-CSF after DCEP regimen is equivalent to mobilize HSC. No difference is observed in the time to take after ASCT. Peg-G-CSF is associated with a better compliance by the patients.

PO-080

BUSULFAN–MELPHALAN (BU-MEL) REGIMEN IN AUTLOGOUS STEM CELL TRANSPLANTATION FOR ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Autologous stem cell transplantation (ASCT) improves the survival of patients with acute myeloid leukemia (AML). In the past, high-dose regimens utilized in ASCT were derived from allogeneic setting. Since these regimens (TBI-Cy, BU-Cy, BU-VP-Cy) provide both cytoreduction and immunosuppression to facilitate the allogeneic engraftment, we evaluated the efficacy of a regimen that thronically gives the maximum therapy to eradicate the disease. Between April 1997 and May 2006, 29 AML patients in Complete Remission (CR) (16 females and 13 males; median age 52 years, range 17-70 with 9 patients >60 years; 9 patients had FAB M1, 9 FAB M2, 8 FAB M4 and 3 had a secondary AML) underwent ASCT. Conditioning regimen consisted of 4d Busulphan (4 mg/Kg from day -5 to -2) followed by Melphalan (140 mg/m²) for 1d (day -1); BU-MEL doses were reduced (3 mg/Kg for 4d and 120 mg/m², respectively) in patients >60 years. Unpurged peripheral blood stem cells in 24 (83%) patients were used, while 5 (17%) patients reinfused bone marrow receiving respectively a median of 3.9x10¹⁰/Kg CD34+ cells (range 0.96-11.5) and 1.45x10⁹/Kg nucleated cells (range 0.29-2.6). All patients achieved full hematological recovery. Median number of days to neutrophil count of 0.5x10⁹/L and platelet count of 20x10⁹/L was 14 (range 12-22) and 18.5 (range 13-80) with no significant differences between the two groups <60 years and >60 years. Transplant related mortality was 3.5%; with 1 patient died for septicemia. As major extra-hematological treatment-related toxicity 21 (73%) patients developed a mucositis episode which was severe (grade III-IV WHO) in 15 of them. There were 16 documented bacterial infections, while 8 patients had fever of unknown origin. After a median follow-up for surviving patients of 30 months from ASCT, 22 patients (74%) are alive and 21 (70%) are in continuous CR. In particular, analysis of two groups <60 years and >60 years showed OS and DFS values respectively of 85% vs 50% and 79% vs 46%. In conclusion, despite the reduced number of patients and the short follow-up, our results demonstrated the feasibility of BU-MEL regimen as conditioning treatment for AML patients who will undergo ASCT also in elderly and secondarily the efficacy of this schedule as evidenced by the high number of continuous CR in patients <60 years. It remains to evaluate the exact role of this approach in patients >60 years, where the acceptable toxicity is not associated with encouraging results.

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11 amyloidosis). In the product of leukapheresis frozeed the mature BFU-E were (mean±SD) 9881±3818x10⁶ while the PBFU-E were 1075±989x10⁶. Only in 12 cases (8 NHL, 1 HL, 8 MM) the PBFU-E outnumbered their mature counterpart (5443.81±3574x10⁶ vs 5370.33±3824x10⁶). Bone marrow transplantation was performed in 36 cases (10 NHL, 4 HL, 21 MM, 1 amyloidosis) with a mean time of transplant from 13.5±2.3 days. PBFU-E were more than mature BFU-E in 5 cases (1 NHL, 1 HL, 3 MM). In this last group the statistical analysis does not confirm that PBFU-E influences the time to take, observed after 12.4±1.1 days from the HSC infusion, whereas was seen after 13.5±2.3 days in the remaining cases (p=0.52). In the multivariate analysis on CD34+ cell count, CFU-GM number, infused mononuclear cell number, PBFE-E and mature BFU-E counts, PBFU-E was the only parameter without statistical significance considering the time needed to reach the take. In conclusion, these data show that the presence of an increased number of primitive BFU-E does not accelerate the take after their infusion of HSC in an autologous transplantation. The statistical analysis indicates that this component is not significant, when compared to the number of CD34+ cells, CFU-GM, mononuclear cells infused, in the hemopoietic reconstitution.

Our data does not show significant difference in all the observed parameters, indicating that the administration of G-CSF or Peg-G-CSF after DCEP regimen is equivalent to mobilize HSC. No difference is observed in the time to take after ASCT. Peg-G-CSF is associated with a better compliance by the patients.
ANEMIAS - APLASIA - THALASSEMIAS

**PO-081**
THE COMPLEMENT INHIBITOR ECULIZUMAB IMPROVES ANEMIA AND REDUCES THROMBOSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) PATIENTS


Naples Federico II, Leeds, Bethesda, Nijmegen, Essen, Homburg/Saar, Helmholtzstr, Melbourne, Baltimore, Paris, Cleveland, Alexion Pharm, Rome La Sapienza, Vicenza S. Bartolo, Milan Osp Maggiore, Firenze, Italy

PNH is a hematologic disorder characterized by the clinical triad of complement-dependent chronic hemolytic anemia, thrombocytopenia (accounting for 45% of mortality) and signs of marrow failure. Hemolysis in PNH is due to the lack of the complement inhibitor CD59 on RBCs, while the life-threatening thrombopathy may be linked to free HB release leading to NO consumption. Eculizumab (Soliris®), an Alexion Pharmaceuticals; EC is a humanized MoAb against the complement fraction 5, which inhibits the terminal MAC formation. EC has been tested in 3 phase III clinical trials, which enrolled 195 patients worldwide. The placebo-controlled TRIUMPH study demonstrated a dramatic blockage of intravascular hemolysis with reduced transfusion requirement. These findings were confirmed in the following open-label SHEPHERD study; thromboembolic (TE) event rate was not a primary endpoint in both studies. We prospectively examined the aggregate TE event rate in EC-treated patients from these 2 trials and a subsequent common Extension study, as compared to each patient's pre-treatment event rate. Before receiving EC, 126 TE events in 195 patients were identified; EC reduced the TE rate in each study. Indeed, the aggregate TE event rate with EC treatment was 1.07 per 100 patient years, compared to 7.37 (p<0.001) in the same patients before EC treatment, corresponding to a reduction of 85% (2 vs 12.3 expected). With restriction of the pre-treatment observation period to the 12 months before initiation of the trials, the TE event rate with EC was reduced 94%, from 17.21 to 1.07, respectively (p=0.002). In patients with TE prior to the trials (n=68), the TE event rate was reduced from 21.42 pre-treatment to 2.27 during EC treatment (p<0.001). Most TE events prior to eculizumab treatment occurred in patients receiving anti-coagulants, indicating that such a treatment may be insufficient to prevent thrombosis. Of 103 patients on anti-coagulants, there were 54 TE events in 30 patients over 385.73 patient years (14.00/100 patient years) pre-eculizumab compared to one TE event with eculizumab (1.07/100 patient years, p=0.001), demonstrating that eculizumab reduces the risk of thrombosis in anti-coagulated highly thrombophilic PNH patients. In conclusion, EC dramatically reduces intravascular hemolysis in PNH patients, resulting in improvement of anemia and of GOL; in addition, long-term EC treatment results in a statistically significant reduction in thrombosis.

**PO-082**
TWO NEW ALPHA-GLOBIN GENE CLUSTER REARRANGEMENTS CAUSING BETA-THALASSEMIA INTERMEDIA PHENOTYPES IN BETA-THALASSEMIA HETEROZYGOUS PATIENTS

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Carriers of beta-thalassemia are usually only slightly anemic. However, a serial of co-inherited factors, either globin gene cluster related or independent, may aggravate the phenotype of the carrier up to transfusion dependency. The effect of alpha-globin gene triplications as a moderate aggravating factor of the mild heterozygous beta-Thalassemia phenotype is known. We describe two cases of simple heterozygosity for the common beta-thalassemia mutation cod59 (C→T), both presenting with a Thalassemia Intermedia phenotype. In both cases synergic effect deriving from membrane defects or red cell enzyme deficiencies were excluded. In one case a triplication of the alpha-globin genes was found which did not justify the severity of the transfusion-dependence phenotype. In both cases multiplex ligation-dependent probe amplification (MLPA) analysis of the alpha-globin gene cluster revealed two new different rearrangements, both resulting into a full duplication of the alpha-globin genes locus. In one case the duplication was in the presence of the common anti alpha3.7 triplication in trans, resulting in a total of 7 active alpha globin genes instead of the normal 4. In the other case the duplicated allele and the normal allele in trans resulted into a total of 6 active alpha-globin genes. Being clinically asymptomatic in the carrier, we postulate that this kind of duplications could be more frequently occurring than expected, explaining at least part of the many unclear cases of severe thalassemia intermedia in beta-thalassemia carriers.

**PO-083**
SAFETY AND EFFICACY OF ECULIZUMAB IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH): SHEPHERD PHASE III CLINICAL STUDY RESULTS


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In PNH, lack of the GPI-anchored terminal complement inhibitor CD59 renders RBCs susceptible to chronic hemolysis, resulting in anemia, fatigue, thrombosis, poor quality of life (Qol) and transfusion dependency. Eculizumab (EC), a terminal complement inhibitor, has shown efficacy in reducing hemolysis and transfusion requirement in a selected PNH population, (TRIUMPH study). To evaluate safety and efficacy of such a treatment, EC was utilized in an open-label non-placebo-controlled phase III study (SHEPHERD) in a broad PNH population including patients with significant thrombocytopenia and/or minimal transfusion requirements. EC was administered to 97 patients at 33 international sites. The most frequent adverse events (AE) were headache (53%), nasopharyngitis (52%), and upper respiratory tract infection (30%). Most AEs were mild to moderate in severity and not considered related to EC; no serious AEs were reported as probably or definitely related to the drug. EC did not increase infections in comparison to the TRIUMPH placebo-arm. Intravascular hemolysis, the primary efficacy endpoint of the trial, was rapidly and significantly reduced, as shown by LDH AUC (p<0.001) and LDH levels (median 2,051 U/L at baseline to 269 U/L at 52 weeks, p<0.001). 8 of 97 patients had incomplete complement blockade and return of hemolysis during the 14-day dosing interval, requiring successful shortening to 12-day interval. Control of hemolysis resulted in improvement in anemia, as transfusion requirements decreased from a median of 8.0 PRBC units/patient during the 12-month pre-treatment period to 0.0 during 12 months of EC treatment (p<0.001). Approximately 50% of the patients were rendered transfusion independent (p<0.001), and hemoglobin levels increased from baseline (p<0.001) during the study. Fatigue levels, as measured by both the FACIT and the EORTC QLQ-C30 instruments, were rapidly and significantly improved with EC treatment (p<0.001). Other EORTC QLQ-C30 patient reported outcomes demonstrated improvement of the global health status, all 5 patient functioning subscales and 7 of 9 symptom/single item subscales. EC appears to be safe and well tolerated. Thus, beneficial effects of EC in PNH were observed in a broader patient population than previously studied, including those with thrombocytopenia and/or minimal transfusion requirements. This further underscores that EC markedly reduces hemolysis, thereby providing significant clinical benefit to treated patients.

**PO-084**
CLINICAL AND HAEMATOLOGICAL FEATURES OF 300 PATIENTS AFFECTED BY HEREDITARY SPHEROCYTOSIS AS A FUNCTION OF THE TYPE OF THE MEMBRANE PROTEIN DEFECT

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Hereditary Spherocytosis (HS) is caused by defects of red cell membrane proteins (spectrin, ankyrin, band 3 and band 4.2). The aim of this study is to analyse a large database of 300 HS patients grouped accord-
were reduced (40%) samples showed VTCR-subfamilies expression. The cellular pat
ners showed blockade of intravascular hemolysis, pointed out by a
ndered by two possible barriers: bone marrow space and immunological
."PO-085
A POSSIBLE NEW CAUSE OF SECONDARY POLYCYTHEMIA: UROLOGICAL CLINICAL
OBSERVATION AND PROPOSAL FOR A CLINICAL TRIAL
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Introduction. One year ago in a polyserositic patient after a prostatic adenectomy (TUR-P) for a large benign prostatic hyperplasia (BPH) (210 ml in volume), the blood cell count became normal after the surgery (1 month) in spite of previously observed need of repeated therapeutic phlebotomies. This observation gave us the idea of a possible new cause of secondary polycythemia since the prostatic cell is able to transform free-Testosteron (FT) in more active Di-Hydro-Testosteron (DHT) and in patients with band 3 and seven spectrin/ankyrin deficiency and also in patients without detectable membrane defect. Furthermore, the sensitivity of all the methods investigated increased in splenectomised cases. AGLT displayed the highest sensitivity, and the association of AGLT and NaCl test on incubated blood reached a sensitivity of 99%, enabling the diagnos-
is of the atypical HS cases, such as those with rare or no spherocytes in blood smears, normal MCHC and reticulocyte counts.

PO-086
IMMUNOLOGICAL TYPEING OF HUMAN EMBRYOS BY CELOCENTESIS TIPIZZAZIONE
IMMUNOLOGICA DEL FLUIDO DELLA CAVITÀ CELOMATICA DI EMBRIONI UMANI
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In utero haematopoietic stem cells transplantation (UH SCT) is hin-
dered by two possible barriers: bone marrow space and immunological tolerance. In human fetus there is evidence of immunocompetence from at least the 11th week of gestation. However, before the 10th week of gesta-
tion it is impossible to carry out UH SCT procedure through intravas-
gal approach. One possible approach is through the
celomic cavity. During the first trimester, by ultrasound transvaginal
approach, one can demonstrate the membrane separating the amniotic
and the celomic cavity, and both fluid compartments could be
studied by ultrasound-guided puncture. Knowledge about cellular com-
position of celomic fluid could be very determinant to know whether
celocentesis could be an opportunity to induce tolerance and chimerism
in fetuses. For this reason we studied the immunological pattern of
human celomic fluids sampled from 6.6 to 10 weeks of gestation. Mater-
ials and Methods. After Hospital Ethical Committee assent, women seek-
ing for voluntary abortion were advised to be included in this study.
Celomic fluid was sampled by ultrasound guided transvaginal puncture.
One sample of celomic cells was used to isolate RNA and performe Vβ,
pre-α and α TCR reverse transcription specific PCR. Cells from sec-
onds were incubated with the specific monoclonal antibodies (CD3+, CD4+, CD105+, CD56+, CD45R0+). Cell lineages. Cellulo-
fluorimetric analysis was carried out using forward scatter/ side scatter and
CD45 gating. Results and Discussion. We studied the celomic fluid
immunological pattern of 17 fetuses from 6.6 to 10 weeks of gestation
for detecting the presence of rearranged VDJβ-TCR transcripts and for
cytotoxicity assay of T, B, NK and mesenchymal cell antigens. 7/17
(40%) samples showed VTCR-subfamilies expression. The cellular pat-
ttern showed a very low frequency of the T, pre-B and B lymphocytes
and NK cells. The high frequency of CD105 suggests that the
mesenchymal/epithelial cells constituted the major cellular population
of celomic fluid. TCR α chain transcripts analysis showed the presence of
only PreT expression, thus indicating the presence of only a pre-T.
The low frequency of the T, pre-B and B lymphocytes antigens suggests
that the celomic cavity could be considered a new route of access to the
fetus for overtaking the immunological barrier to IUH SCT engraftment
or donor specific tolerance.

PO-087
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) IN THE ECULIZUMAB ERA: THE
BEDSIDE AND BEYOND
Ristorino AM1, Hill A2, Ricci P2, Selleri C2, Marando L3, Gianfaldoni G2, Mannelli F2, Milano E2, Amendola A1, Boschetti C1, Di Bona E4, Barbano F2, D’Amico P1, Rodighiero F2, Zanella A4, Iori AP3, Notaro R2, Rother RP, Kroon HA, Hillmen P2, Luzzatto L1, Rotoli B2
1*Naples Federico II, 2 Florence ITT and University; 3Lecce, Rome La Sapienza, 4Mila
Osp. Maggiore, 5Vicenza S. Bartolo, 6 S. Giovanni Rotondo, Italy 7Alex-
in Pharmaceuticals, US
PNH is characterized by complement-mediated chronic intravascular
hemolysis due to the lack of the complement regulators CD55 and CD59
on the RBC surface. Eculizumab (Soliris®), Alexion Pharmaceuticals; EC)
is a humanized monoclonal Ab against C5; which inhibits the terminal
MAC formation. We have collected clinical and experimental data from
21 Italian transfusion-dependent PNH patients enrolled within the EC-
based international trials TRIUMPH, SHEEPERD and Extension. All
patients showed blockade of intravascular hemolysis, pointed out by a
not only for a brain-storming but to observe in polycythemic patients, first
of all, those who underwent repeated phlebotomies if large BPH is con-
current and TRUS with prostatic volumetry and DHT dosage performed.
Eventually, we will be grateful for any support for a scheduled trial to be
discussed during the meeting.
PO-088
NEW INSIGHTS IN THE FA CELLULAR PHENOTYPE
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We have studied chromosomal instability (CI) in a cohort of 94 Fanconi Anemia (FA) homozygotes, 63 FA heterozygotes and 501 non-FA subjects. CI was scored by the same cytogeneticist and revised in blind by two additional cytogenetists. Spontaneous CI displayed a significant gender difference, with a male:female ratio of 1.5 (alpha = 0.00026), unlike non-FA subjects where the male:female ratio was close to 1. It may be suggested that CI might also depend on factors different from the specific genetic defect, such as hormonal status and FA-associated oxidative stress. This finding is consistent with a greater mortality in female respect to male patients. This evidence gives an implication on mosaicism definition, currently based on FA patient’s CI only and rated independently of the gender, then on clinical management of FA patients, which changes if the patient is defined as a mosaic. Moreover, we report the application of multi-colour fluorescence in situ hybridization by Spectral Karyotyping (SKY - ASI - Israel) for FA chromosome rearrangement analysis. This multi-painting method proved advantageous in detecting and identifying the chromosome changes in FA metaphase cells that were unresolved or undetected by conventional cytogenetic analysis. This technique is less dependent on chromosome morphology, allowing the analysis of cells unsuitable for conventional methods. The results obtained by this approach show a random chromosome involvement in rearrangements, while some chromosomes seem to be preferentially involved in breakages. This non-random involvement of some chromosome regions suggests the existence of a hierarchy in genetic aberrations of FA cells but their significance is far from clear and needs further investigation.

PO-089
A NEW BETA-CHAIN HAEMOGLOBIN VARIANT: HB ROMA [BETA115 (G17) ALA->VAL]
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Here we describe a new haemoglobin (Hb) variant, due to a GCC>GTC transition at codon 115, which results in an Ala->Val amino acid substitution in the beta chain. This Hb variant, never reported before in the literature, was named Hb Roma. All separation techniques we tested, including electrophoresis on citrate agar (pH 6.0) and on cellulose acetate (pH 6.0) and cation exchange HPLC (Variant II™, Bio-Rad Laboratories, Hercules, CA, USA) did not reveal any abnormal mobility. The isopropanol stability test, on freshly prepared red cell lysate, was positive. The alpha/beta globin biosynthesis ratio, obtained by reversed phase HPLC, using a Vydac large pore C4 column (the Separation Group, Hesperia, CA, USA) was >2.0. The molecular defect was characterized by direct DNA sequencing of the beta-globin gene (Figure 1) and confirmed by a designed ARMS-PCR protocol. Haematological studies were carried out by standard methods and the haematological parameters, with the haemoglobin typing, are described in the Table 1.

Table 1. Haematological parameters and haemoglobin typing in the Hb roma carrier.

<table>
<thead>
<tr>
<th>Sex</th>
<th>age (yrs)</th>
<th>RBC (10^12/L)</th>
<th>Hb (g/dL)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>Hct (L)</th>
<th>Ret (g/L)</th>
<th>PLT (10^9)</th>
<th>ESR (mm/h)</th>
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<td>24.9</td>
<td>1.61</td>
<td>0.34</td>
<td>25</td>
<td>6.73</td>
<td>0.6</td>
<td>2.7</td>
<td>4.0</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. beta-globin dna sequence of the hb roma carrier, showing the gcc->gtc point mutation at codon 115. The left panel shows the forward sequence. The right panel shows the reversed sequence.

The proband presented a light beta-thalassaemia phenotype with mild hypochromia and microcytosis. Direct sequencing of the entire beta-globin gene revealed only the described new mutation. The presence of the most common alpha-globin deletionalthalassemic mutations and of the alpha alpha anti 3.7 allele have been excluded as well. Therefore, the only presence of this Hb variant in a heterozygous state leads to the slightly altered haematological condition and to the mild instability in the presence of isopropanol. On the other hand, differently from other unstable Hb variants, the Hb Roma does not cause in the patient the phenotype of an haemolytic anaemia with reduction of the Hb level or reticulocytosis associated with splenomegaly. Finally the alpha/beta globin biosynthesis ratio results particularly high (>2.0), not in correlation with the haematological and haemoglobin features of the patient but more suitable for an intermediate thalassemia. Thus further functional and molecular studies on the patient and her family will be of pivotal importance to correctly characterize this Hb variant.

PO-090
EFFECT OF HYDROXYUREA ON EXTRAMEDULLARY HAEMATOPOIESIS IN THALASSEMIA INTERMEDIA: CASE REPORTS AND LITERATURE REVIEW
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Extramedullary haematopoesis (EH) is the outside bone marrow...
production of blood cells precursor. EH occurs in haemolytic anemias as a physiological response to increased erythropoietin. On the other hand, in the setting of polycythemia vera, myelofibrosis or chronic myelogenous leukemia EH is due to a clonal disorder causing the escape of hematopoietic cells with lodging into other organs. EH sometimes can lead to symptomatic tumor-like masses. We describe intrathoracic and symptomatic pelvic EH masses in two patients affected by thalassemia intermedia. Case 1: A 48-year-old woman was admitted to our Centre in January 2004. She was previously transfused with 500 ml of packed red cells three to four times a year from 9 to 22 years of age with pre-transfusion haemoglobin levels of 7.8 g/dL. From 1979 she stopped transfusions and her haemoglobin levels remained at 8.8 g/dL. In 1988 an asymptomatic pelvic mass of 12 cm of diameter was documented by abdominal echography. She underwent splenectomy in 1988. During splenectomy a pelvic mass biopsy led to the diagnosis of extramedullary haematopoesis mass but no therapeutic actions were taken. The patient came to our attention in 2004 because of abdominal pain and diarrhoea, pollakiuria, MRI showed a 12×13 cm pelvic lesion, with lobular shape and hemorrhagic centre; it arose in Douglas pouch, making compression on uterus and bladder on rectum and left ureter with pyelo-ureteral enlargement in back and upper side. In February 2004, the patient started therapy with Hydroxyurea (HU) and sporadic transfusion support. HU was given at dose of 15 mg/Kg/die, for one month with HU therapy. Case 2: Is a 42 years old man, with diagnosis of B thalassemia intermedia, (IVS 1-110/βthalassemia). He required transfusions two or three times a year in early childhood to maintain a steady state haemoglobin level of 8.5-9.0 g/dL. In 1998 a routine chest X-ray showed bilateral mediastinal masses. In June 2001 the patient developed bilateral pleural effusions requiring thoracentesis and pleurodesis. MRI supported the hypothesis of thoracic EH. The patient was treated with HU with a good clinical and MRI response. The mechanism of HU in supporting the hypothesis of thoracic EH. The patient was treated with a combination of packed red cells three to four times a year from 9 to 22 years of age with pre-transfusion haemoglobin levels of 7-8 g/dL. From 1979 she stopped transfusions and her haemoglobin levels remained at 8-8.5 g/dL. In 1988 an asymptomatic pelvic mass of 12 cm of diameter was documented by abdominal echography. She underwent splenectomy in 1988. During splenectomy a pelvic mass biopsy led to the diagnosis of extramedullary haematopoesis mass but no therapeutic actions were taken. The patient came to our attention in 2004 because of abdominal pain and diarrhoea, pollakiuria, MRI showed a 12×13 cm pelvic lesion, with lobular shape and hemorrhagic centre; it arose in Douglas pouch, making compression on uterus and bladder on rectum and left ureter with pyelo-ureteral enlargement in back and upper side. In February 2004, the patient started therapy with Hydroxyurea (HU) and sporadic transfusion support. HU was given at dose of 15 mg/Kg/die, for one month with HU therapy. Case 2: Is a 42 years old man, with diagnosis of B thalassemia intermedia, (IVS 1-110/βthalassemia). He required transfusions two or three times a year in early childhood to maintain a steady state haemoglobin level of 8.5-9.0 g/dL. In 1998 a routine chest X-ray showed bilateral mediastinal masses. In June 2001 the patient developed bilateral pleural effusions requiring thoracentesis and pleurodesis. MRI supported the hypothesis of thoracic EH. The patient was treated with HU with a good clinical and MRI response. The mechanism of HU in reducing the masses of EH is not yet completely known. HU, stimulat
tion were absent, while s-EPO was still high without any sign of respiratory or cardiovascular pathology. We supposed a secondary erythrocytosis due to an Hb variant and the patient was referred to Centro Studi Microteicemie of Rome where a sequence analysis of beta-globin gene on the amplified DNA demonstrated the hexozygosity for a CGT->ATG mutation at codon 20 (Val->Met) corresponding to the Hb Olympia firstly reported by Stomatoyannopoulos. This Hb has high O2 affinity leading to erythrocytosis. The presence of Hb Olympia and an hemolytic anemia due to HS in the same patient is highly suggestive. Before the splenectomy, the hemolysis was the main clinical feature although there was not a relevant anemia. The haemolytic rate was very high as indicated by splenomegaly and bilirubin level. An high compensation rate was present too (high reticulocytes and s-EPO). Nevertheless the HS without anemia are not rare. The splenectomy removed the hemolysis and the presence of Hb Olympia became evident.

PO-094
A DISCRIMINANT FUNCTION TO EVALUATE HYPOCHROMIC ERYTHROCYTES.
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Hypochromic erythrocyte percentage (%HE) is used to diagnose and follow up iron deficiency (ID). Recently, it has also been proposed as a marker of available/utilizable iron. Micoscopic evaluation is now replaced by parameters obtainable by automatic counters (Hb, Bayer; ADVIA-120, Bayer, etc.), although not all counters can perform a direct count (e.g.: XE-2100, DASIT). As in ID RDW-SD is increased, MCH is reduced and the equivalence Ht=Hb/x3 is missed, we tried to build a discriminant function (DF%HE) to evaluate %HE also by those autoanalysers which do not provide directly this parameter. 450 adults, both M and F, subdivided in two cohorts of 300 and 150 subjects, were studied. The first cohort was composed by 100 anemic microcytic subjects, either iron-deficient or heterozygous thalassemics, with Hbx3<Ht, and 200 controls with Hbx3=Ht. The second cohort was composed by 100 hypochromic subjects with %HE between 10-70% and 50 normocytic normochromic controls with %HE<10%. Two autoanalysers (Hb, that directly counts %HE, and XE-2100 that does not perform this evaluation), a HPLC device to evaluate the percentage of HbA2 and conventional microscopy were employed. Hypochromic subjects were divided into four ranks: 1°) 10-20%; 2°) 20-30%; 3°) 30-40%; 4°) >40%. Four of these subjects have been followed by serial controls. Statistical analysis was performed by an XP-6 software and linear regression according to Altman. DF%HE was calculated as follows: DF%HE=[Ht – (Hbx3)+1] x (RDW-SD/MCH)x3. %HE was evaluated by Ht and by XE-2100 in the first cohort of patients; microscopic examination and DF%HE were evaluated in the second cohort of patients. Peripheralsmears of subjects belonging to the second cohort were stained by MGG; each sample was evaluated by counting HE in different microscopic fields; %HE was calculated as percentage of total erythrocytes. The values of %HE obtained by DF%HE were not significantly different from those obtained by both calculated H3 analyser and those calculated by the microscopy. The evaluation of the four ranks showed increasing SD in parallel to %HE increase. The analysis of the four patients serially evaluated showed that age, transfusions and dialysis do not alter sensitivity and specificity of this method. We think that DF%HE allows a reliable count of %HE also by those analysers which cannot perform directly this evaluation. DF%HE can be easily added to the software of any analyser without further expenses.

PO-096
PURE RED-CELL APLASIA (PRCA) DUE TO PARVOVIRUS B19 IN A CASE OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) TREATED WITH RITUXIMAB AND ALEMTUZUMAB
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The case refers to a 48-year-old man with a 3–years history of B-CLL at Binet stage B. Since his disease was refractory to first-line therapy (6 courses of Fludarabine and Cyclophosphamide), he was treated with 6 courses of Rituximab-CHOP followed by Alemtuzumab given subcutaneously at a total dose of 15 mg 3 times per week for 15 weeks. At the end of the treatment a complete clinical response with bone marrow and peripheral blood purging was obtained. Subsequently he was treated with Cytarabine (800 mg/m2) every 12 hours for 6 doses, followed by subcutaneous G-CSF for peripheral blood stem cell collection. One month later, the patient presented a severe transfusion-dependent Coombs-negative anaemia, reticulocytopenia and selective deficiency of erythroblasts in an otherwise normal marrow aspirate. The bone marrow biopsy showed the presence of giant pronormoblasts with cytoplasmic vacuolization and inclusions suggesting a Parvovirus B19 infection, confirmed by elevated levels of anti-Parvovirus IgM antibodies and polymerase chain reaction (PCR). The patient has been transfused with 13 packed red cells units. After the diagnosis of parvovirus B19 infection, intravenous immunoglobulin (IVIG) (0.4 g/kg over 5 days) was administered: 8 days later reticulocytosis and no longer need for transfusion were recorded. Moreover 16 days after Parvovirus IgM and PCR were negative, PRCA was a rare disease. Acute acquired PRCA is usually caused by viral infections as Parvovirus B19. In the normal host Parvovirus infection can be asymtomatic, while the immunocompromised patients (acute/chronic leukemias, lymphomas, AIDS) are at risk for symptomatic PRCA. Alemtuzumab is an anti-CD52 monoclonal antibody effective for the treatment of CLL, but causes prolonged severe CD4+ and CD8+ depletion. Likely, this additional immunodeficiency has favoured the development of PRCA in our patient already immunodepressed by CLL and its previous treatments. Rare cases of Parvovirus B19-related anemia worldwide. Diagnosis is commonly based on evaluation of Hb, serum iron, ferritin and/or transferrin saturation (TSAT). In the quest of further confirmatory methods of diagnosis, several other diagnostic parameters have been proposed, such as the percentage of hypochromic erythrocytes (%HE), serum transferrin receptor (sTfR), reticulocyte hemoglobin content (CHR), zinc protophorphyin (ZnPPE), etc. Taking into account the established role of %HE in the diagnosis of iron deficiency and the fact that in iron deficiency zinc replaces iron in heme group producing zinc protophorphyin, we tried to increase the discriminatory power of these two parameters in the diagnosis of microcytosis by the construction of a function named zinc protophorphyin-equivalent: ZnPPE=%HE x 30/Hb. This function was tested in a wide cohort of patients and was put in correlation with other diagnostic indexes. 1648 subjects were studied: 976 between 6 and 10 years of age and 704 adults. 1087 normal subjects were controls (788 children and 299 adults). 186 anemic children were represented by 155 iron-deficient anemics, 18 non-iron-deficient anemics and 15 heterozygous beta-thalassemics. 375 anemic adults were divided in micro-, normo- and macrocytics or hypo-, normo- or hyperchromics. Peripheral blood samples were used to obtain common erythrocyte and reticulocyte parameters, ferritin, serum iron, transferring, ESR, CRP. The following indexes were evaluated: ZnPPE, CHR, erythroide bone marrow activity (EBMA) and efficiency (EBME). Instruments employed were: H5(Bayer), ADVIA-120(Bitachi), Hitachi 747(Boche), ALA-120(Tosoh), VHCA-47(Rohde), VA-817(Tosoh). The analysis was performed by XP-5 software. In children, ZnPPE was increased in all cases of iron-deficient anemia but not in thalassemia. In adults, ZnPPE was increased in iron deficiency, functional iron deficiency (iron blockade by chronic inflammatory), and in thalassemia. ZnPPE seems to be a specific and sensitive index to identify microcytic anemia due to iron deficiency in children, although it cannot discriminate between true and functional iron deficiency and between iron deficiency and thalassemia in adults. At variance with ZnPPE, whose evaluation implies dedicated analyser, ZnPPE can be calculated through parameters obtainable by a simple hemogram.
PRCA have been reported in patients receiving monoclonal antibodies and IVIG administration appears to be the only effective treatment in such cases. Consequently we made our decision to additionally look for Parvovirus B19 infection in patients treated with monoclonal antibodies who develop agranulocytic anaemia. In order to prevent PRCA in the future, we think it is advisable to administer IVIG prophylactically, in immunocompromised patients treated with Alemtuzumab or Rituximab.

**PO-097**

**GELATINOUS DEGENERATION OF BONE MARROW: REPORT OF TWO CASES WITH DIFFERENT HAEMATOLOGICAL FEATURES AND CLINICAL OUTCOME**


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Gelatinous marrow transformation (GMT) is a rare histological disorder of unknown pathogenesis characterised by deposition of seromucinous gelatinous material in the bone marrow (BM) stroma. It has been reported in association with chronic debilitating diseases, such as anorexia nervosa, malnutrition, HIV infection and treatment-related cytotoxic BM injuries. In patients with haematological malignancies, this complication has been described in few cases of acute myeloblastic leukaemia with monosomy 7 and only exceptionally in those affected by other neoplastic disorders. We recently observed and hereby present two additional cases of GMT not associated with previously reported conditions and characterised by different haematological features and clinical outcomes. The first case was a 64 years old Caribbean woman, living in Rome from more than 30 years, who was kept under our attention in December 2005 because of an isolated normocytic anaemia. Her past medical history was not noteworthy. An isolated normocytic anaemia was the only pathological finding. Her nutritional status was very good. BM aspiration was repeatedly unsuccessful (dry tap); therefore, a BM trephine biopsy was performed and histological pictures of gelatinous degeneration (Figure 1) were found.

**Figure 1.** Bone marrow trephine biopsy. Massive necrosis and an overall feature of ghost-like cells (A). High Magnification – 40x – revealed necrotic cells with darkly staining pyknotic nuclei as well as kariorrhexis and karyolysis (B).

The patient received several treatments, including erythropoietin, prednisone and cyclosporine without any benefit. To date, with a follow up of 24 months, her haematological status is stable and she is managed with supportive therapy alone (2 red blood cells packets every 7-10 days). The second case was a 65 years old man who was diagnosed in November 2005 as having a myelodysplastic syndrome (MDS) with monosomy 7 for which he received several treatments without any benefits. Three months later, his medical status worsened and a progressively severe pancytopenia developed. BM aspiration failed, resulting in a dry tap for which trephine biopsy was performed and a diagnosis of GMT was made. Despite several treatment attempts, the patient deceased soon after because of severe complications related to his BM failure. Our report provides two additional cases of GMT, with very different haematological features and clinical outcome, concerning an otherwise healthy woman and a MDS patient. Therefore, GMT represented the not specific pathological finding associated with different underlying conditions, which clinical expression varied from a long-lasting and isolated anaemia to a rapidly developing BM failure and pancytopenia, reflecting these findings the implication of basic bioregulatory processes in its pathogenesis. Indeed, the hyaluronic acid deposition, which represents the pathological hallmark of this condition, probably inhibits haemopoiesis by altering the haematopoietic microenvironment and the BM stroma and possibly hampering the interactions between cells and cellular signalling.

**PO-098**

**ACUTE BONE MARROW NECROSIS AS TERMINAL COMPLICATION OF A VERY LONG-LASTING POLYCYTHEMIA VERA**


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Bone marrow necrosis (BMN) is a rare complication of a variety of malignant solid and haematological disorders, infections and drug-induced toxicities. BMN is morphologically characterised by the destruction of haematopoietic tissue, including the stroma. Major clinical features are an abrupt onset, elevated fever and severe and generalised bone pain. Pancytopenia and embolic processes often involving the lungs are major complications of BMN. Elevated serum lactatedehydrogenase (LDH) and alkaline phosphatase are the most common laboratory markers. BMN should be managed with supportive therapy and its prognosis reflects the outcome of the underlying disease. To the best of our knowledge, this complication has not been described in patients with polycythemia vera (PV), as recently observed by us. A 71 year old man was seen on February 2007 because of general malaise, hyperpyrexia and severe, diffusse bone pain.

In 1992, he was diagnosed as having PV and was treated for several years with pipobroman followed by hydroxyurea (HU), withdrawn three months before the admission because of leg ulcers. Five days before the admission, BM aspiration (dry tap) and a trephine biopsy had been performed because of a monocytosis and a transfusion-dependent anaemia; BM was hypercellular with 22% of fine granulated blasts, so that a diagnosis of acute myeloid leukaemia (AML) was made. On examination, the patient was ill-looking, febrile and pale. Physical examination revealed massive splenomegaly, moderate hepateomegaly and lower limb oedema. Radiological work-up, including a total body CT scan, showed a limited bilateral pleural effusion but no other abnormalities. The laboratory work-up showed haemoglobin (Hb) concentration of 9.0 g/dL, a white blood cell (WBC) count of 23,200×10^9/L, and a platelet count of 750,000×10^9/L. His liver function tests and renal function tests were only slightly abnormal. Septic and virology work-up was negative and no diagnostic signs of disseminated or localised or mycotic infections were found. Cold agglutinins and mycoplasma antibodies were negative. Moreover, no clinical or laboratory feature of autoimmune diseases were revealed. The examination of cerebrospinal fluid revealed no abnormality. Broad spectrum antibiotics and Amphotericin B were started without any benefit. In addition, methyl prednisolone was empirically started, covering the possibility of vasculitis. Five days after the admission, his general condition worsened and progressively more severe pancytopenia developed; serum LDH and alkaline phosphatase raised to 8900
U/L (< 460) and to 1298 U/L (90-360) respectively. A trephine biopsy could be eculizumab or a mini allogeneic stem cell transplantation.

"A RARE CASE OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) FOLLOWING AUTO STEM CELL TRANSPLANTATION FOR NON-HODGKIN LYMPHOMA"

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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is an uncommon form of haemolytic anemia. It results from the clonal expansion of hematopoietic stem cells that harbor a mutation in the PIG-A gene leading to a deficiency of glycosylphosphatidylinositol (GPI) membrane-anchored proteins. Methods. A 52-year-old man developed PNH after auto stem cell transplantation (ASCT) for non-Hodgkin lymphoma (NHL). He was admitted for stage IV A (M1), FLIPI 2, grade III follicular lymphoma, and received 6 cycles of R-CHOP chemotherapy, obtaining a complete remission (CR). After 5 months he developed a relapse and he started a MIMA scheme then high dose sequential chemotherapy with vePesid, cyclophosphamide, aracrin and four doses of rituximab, finally followed by melphalan (180 mg/m²) and mitoxantrone (60 mg/m²) supported by ASCT. After ASCT the persistence of mild pancytopenia was observed; biopsies showed multilineage dysplasia without evidence of recurrence of NHL. Results. After 11 months from ASCT the patient referred increasing anemia, esphaghal spasms and emission of red urine. Laboratory data showed: WBC 2.8 × 10⁹/L; RBC 4.03 × 10¹²/L; hemoglobin 10.6 g/dL, reticulocytes 5% (14×10⁶/L), platelets 48×10⁹/L; Vitamin B12, folate and erythropoietin level were 155 pg/mL (NV 208-964), 5.3 ng/mL (3-17.5) and 82 IU/mL (4-35) respectively. Liver enzyme levels were elevated, later increasing until ten time the normal values. Anemia worsened, haptoglobin was <0.06 g/L, LDH about 5000 U/L; direct and indirect Coombs tests were negative, while urine was highly positive for haemoglobin. Erythropoiesis have a relevant IOV also if not transfused. This fact should be considered for therapeutic approach. Moreover, T2° is useful in differentiating the HH from the DMS patients.

Table 1.

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PO-101
HEPATIC IRON CONCENTRATION INTRALIVER VARIABILITY IS INFLUENCED BY BIOPSY SAMPLES’ QUALITY

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Background. Hepatic iron concentration (HIC) is widely used in research practice and in clinical research. However HIC variability among biopsies have been reported in several condition. The aim of this study was to evaluate intraliver hepatic iron concentration variability. Methods. We determined HIC by percutaneous liver-biopsy under ultrasound guidance in 75 iron overloaded patients, 60 patients who had undergone bone marrow transplantation for thalassemia major and 15 patients affected by thalassemia major. Two liver biopsy specimens were taken from each patient using the same cutaneous access inclining the needle in opposite directions into the right liver lobule. Degree of fibrosis was evaluated using Ishak classification: 6 patients had no fibrosis at all, 15 showed minimal fibrosis, 22 moderate fibrosis, 18 severe fibrosis, and 14 were affected by cirrhosis. Considering biopsy specimens’ degree of fibrosis and the hepatic iron concentration (in milligrams per gram of liver, dry weight) patients were identified two groups: Group 1:14 patients with cirrhosis; Group 2: 61 patients without cirrhosis and both samples > 1 mg of dry weight. Results. The variance of the residual values from the regression model for samples revealed a significant correlation between the two different specimens’ dry weight among all 75 patients (r > 0.959; p<0.01). The coefficient of determination, calculated to estimate the proportion of variation is r²=0.828 (p<0.01) in patients without cirrhosis and one or both samples <1 mg of dry weight and is r²=0.896 (p<0.01) in patients without cirrhosis and both samples > 1 mg of dry weight. Conclusions. Intraliver variability exists since liver iron concentration was influenced by the weight of the liver sample obtained at biopsy and presence of cirrhosis. These data demonstrate that such a variability is significantly inferior in the liver-biopsy samples that are at least 1.0 mg in dry weight and without cirrhosis.
ALLOGENEIC TRANSPANTATION

PO-102

IMMUNE RECONSTITUTION OF THE T CELL COMPARTMENT IN MULTIPLE MYELOMA PATIENTS FOLLOWING ALLOGENEIC NON MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION


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Introduction. Allografting is the only potentially curative treatment for multiple myeloma (MM). However, relapse and treatment-related toxicity are major obstacles to cure. Reduced-intensity/non-myeloablative conditioning regimens were designed to initially establish hematopoietic mixed chimerism and then to serve as a platform for additional cell immunotherapy aimed at eradicating tumor cells in elderly patients (up to 70 years). However, the risk of post-transplant infections and graft-versus-tumor effects also rely on the residual thymic function, gradually reduced with age. For this reason it is mandatory to evaluate the residual thymic function in this group of elderly patients. Naive CD4+CD62L+CD45RA+bright T cells were evaluated by flow cytometry in 38 MM patients, median age 55 years (range 34-64), conditioned with low dose TBI (200 cGy), with/without fludarabine (90 mg/m² total), followed by G-CSF mobilised peripheral blood stem cell infusion from HLA identical siblings. The analyses were performed at different time points: baseline, at day +28, at 3, 6 months, and at 1, 2, 3, 4, 5, 6 years post-transplant. Briefly, fresh peripheral whole blood samples were stained with direct four-colour combinations of the following MoAbs: CD3, CD4, CD8, CD16, CD45RA, CD45R0, CD62L. At least 80000 events for each combination were acquired on a FacsCalibur (Becton Dickinson), and analysed with CellQuest Pro software. T cell Receptor Excision Circles (TRECs) were evaluated by real-time quantitative PCR with an ABI PRISM 7900HT Sequence Detection System at the same time points. Results. CD4+ T cell >200/uL promptly recovered by day +28 with median values of 272/ microliters, gradually increasing to 466/microliters, 605/microliters, 889/microliters, and 953/microliters at 1, 2, 4, 5 and 6 years, respectively. Naive CD4+CD45RA−/bright T cells increased to 49/microliters, 65/microliters, 102/microliters, 125/microliters, at day +28, and at 1, 5, 5 years, respectively. Memory CD4+CD45R0−/bright remained stable with median values of 153/microliters and 158/microliters by day +28 and 3 months; respectively; then increased to 224/microliters, 378/microliters, 523/microliters, at 1, 2, 4, more then. Moreover, the evaluation of the coexpression of the CD45 isoforms showed that the number of CD4+CD45RA−/bright T cells reached median values of 65/microliters by day +28 and 79/microliters at 6 months; then increased to 140/microliters, 192/microliters, 280/microliters at 2, 4, 5 years, respectively. CD8+ T cells reached median values of 147/microliters by day +28, increasing to 330/microliters, 750/microliters, and 1021/microliters at 3 and 6 months, and at 4 years, respectively. CD4/CD8 ratio was 1.8 by day +28, decreased to 0.5 at 6 months, and remained low at 0.75 and 0.9 at 2 and 5 years, respectively. In a subset of 24 patients the presence of naive CD4+CD62L−CD45RA−/bright T cells and of memory CD4+CD62L−CD45R0−/bright T cells was evaluated. Preliminary data showed an increase of these cell populations at 3 years with a median number of 924/microliters, and 80/microliters respectively, while they remained stable at 5 and 6 years. TREC copies/100ng DNA of PBMC were measured in 32 patients at the same time points: median baseline value was 0.25, then they gradually increased to 4.3 at 1 year, and reached 15.6 and 45.7 at 3 and 5 years. A significant correlation was demonstrated between TREC values and CD4+CD62L−CD45RA−/bright T cells (p<0.0001). Conclusions. Our findings suggest that the immune reconstitution following allogeneic transplantation that differs from normal T lymphocyte ontogeny. Preliminary results show a significant correlation between the quantitative analysis of TREC's and the analysis of naive T cells by CD62L expression and will allow to more precisely quantify the residual thymic function in this group of elderly patients.

PO-103

A RAPID CLEARANCE OF MRD AFTER ALLOGENEIC STEM CELL TRANSPLANTATION PREDICTS A FAVOURABLE OUTCOME IN HIGH RISK ADULT ALL PATIENTS

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Introduction. Allogeneic stem cell transplantation represents a curative option for patients with acute lymphoblastic leukemia (ALL) with high-risk features at diagnosis. Aim of this study was to correlate the kinetics of MRD clearance after allogeneic transplantation with the clinical outcome. Methods. MRD was evaluated by RQ-PCR using probes derived from fusion chimeric genes (BCR/ABL and MLL/AF4, n=22) or rearrangements of the T-cell receptor or the Immunoglobulin genes (n=21). Patients were eligible to allogeneic transplantation in first CR only if they had adverse cytogenetic abnormalities including t(9;22) or t(4;11) or persistent MRD during consolidation chemotherapy. Results. The median follow-up was 85 months (range 3-203). In 28 patients the presence of naive CD4+CD62L+CD45RA+bright T cells was documented so that 71% of patients converted to a molecularly negative status. At day +100 a significant 3 log mean reduction was confirmed in the BM but evidence of leukemia persistence/progression was documented in 44%. With a median follow-up of 28 months (range 4 - 138), the OS at 36 months of these patients was 48%. The OS of CR patients was 80% for those who proved PCR negative before transplant as compared to 49% for PCR positive patients (p=0.17). The cumulative incidence of relapse was 0% for PCR negative patients and 46% in MRD positive patients (p=0.027). Moreover, the relapse rate of patients with PCR negativity at day +100 after transplantation was remarkably low (7%) as compared to patients who proved PCR positive (80%, p=0.0006). By multivariate analysis, only the molecular CR before conditioning proved to be a significant predictor for the achievement of a molecular negativity at day +100 after transplantation. The molecular CR was 100% in all patients with a molecularly detectable residual disease, before the conditioning regimen. This observation underlines the need of therapeutic programs which can increase the proportion of molecular remission before transplantation. Furthermore, patients who did not achieve PCR negativity by day +100 post transplant, showed a significantly higher relapse rate compared to patients who achieved a molecular remission at this early time point. Our results suggest that the check point at day +100 is crucial for subsequent decisions aimed to preventing leukemia relapse such as modulation of immune suppressive drugs, infusions of donor lymphocytes or new experimental therapeutics.

PO-104

NATURAL KILLER CELLS EXPANDED UNDER GOOD MANUFACTURING PRACTICE CONDITIONS EXERT LYTIC ACTIVITY AGAINST ACUTE MYELOID LEUKEMIA BLASTS


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Natural killer (NK) cells have been demonstrated capable of controlling the leukemic expansion in experimental models and are known to play a key role in the context of haploidentical KYR mismatch stem cell transplantation (SCT). We recently demonstrated that NK cells can be greatly expanded both from normal donors and from leukemic patients and we verified the capacity of this population of expanded effectors to recognize and kill allogeneic and autologous primary myeloid and lymphoid leukemia blasts. Aim of this work was to verify whether NK cells can be expanded from the peripheral blood of normal donors under Good Manufacturing Practice (GMP) conditions and whether these expanded effectors may potentially exercise anti-leukemic activity. This information may indicate possible new therapeutic strategies for the management of minimal residual disease (MRD), also in the setting of allogeneic SCT. We investigated the peripheral blood of 17 adult donors.
Enriched NK cells were expanded for 14 days at 37 °C in the presence of infusing into a leukemic patient with evidence of MRD after an allogeneic SCT an amount of 1-7 × 10^6 NK cells/kg of body weight. The infusion of NK cells should induce very limited toxicity and no or very low graft-versus-host disease, thus avoiding the potential complications associated to donor T-lymphocyte infusions and making the design of NK cell-based clinical trials for the management of allografted AML patients a feasible option.

**PO-105**

**Autoimmune hemolytic anemia in recipients of allogeneic cell depleted hematopoietic peripheral blood stem cell transplants**


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**Methods.** The incidence of autoimmune hemolytic anemia (AIHA) was assessed retrospectively in patients who received allogeneic T-cell-depleted transplants. Between January 1999 and December 2006, 326 patients have been transplanted from either HLA-matched (n=102) or HLA-mismatched donors (n=224). AIHA was defined on the basis of (1) a positive direct or indirect Coomb’s test; (2) positivity of at least two of the following clinical parameters: decreased haemoglobin, high reticulocyte count, increased bilirubin levels, reduced haptoglobin levels. Hemolysis due to Rh or ABO incompatibility was excluded from this data analysis. **Results.** 29 of the 326 patients (8.9%) developed AIHA, at a median of 7 months post transplant (range 4-22 months). The incidence overlapped in transplants from HLA-matched (9/102) and mismatched (20/224) donors (8.8% vs 8.9%). Hemolytic anemia was due to hot IgG (Coomb’s direct test positivity) in all cases, and to C3d positivity in 23. Coombs indirect test was positive in 26/29 patients; with panreagent serum in 23/26; anti-C + D specificity in 2 and anti-E specificity in 1. 19/29 patients had concomitant, recurrent CMV infection; 2/29 with grade II acute GVHD were under steroid therapy. Therapeutical strategies were: a) 1 patient, polyspecific immunoglobulins which resolved hemolytic anemia; b) 13 patients, polyspecific immunoglobulins followed by one or two cycles of Rituximab, according to response. Five patients achieved remission; in 4 the remission was due to immunotherapy (infusion of donor CD3 lymphocytes). Five patients achieved remission, 2 partial remission and 8 was resistant. Hemolysis was resistant in 14 patients. Three underwent splenectomy, 6 received steroid and/or cyclophosphamide therapy and 3 underwent both splenectomy and immunosuppressive therapy. Overall, 20/29 (69%) patients responded to therapy while 9/29 (31%) were resistant. One of the 9 non-responders died 10 days after the onset of AIHA; the others achieved stable haemoglobin levels with positive hemolysis indices. **Conclusions.** The incidence of AIHA was about 9% in our T-cell-depleted transplants. In absence of GVHD, hemolytic anemia was mainly associated with recurrent CMV infection. Most cases responded to immunoglobulins and Rituximab. Splenectomy was rarely required. Hemolysis did not increase TRM.

**PO-106**

**The effect of allogeneic stem cell transplantation on outcome in cytogenetically normal acute myeloid leukemia patients and correlations with PGP expression**

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**Introduction.** Cytogenetically normal (CN) acute myeloid leukemia (AML) patients are included in an intermediate risk group, with a median 5-year overall survival (OS) of 85%, but they display an heterogeneous clinical course. Several genetic abnormalities seem to impact on the prognosis of these patients and we recently demonstrated that P-glycoprotein (PGP) overexpression is related with a reduced survival also in CN AML patients. Allogeneic stem cells transplantation (SCT) is recommended in patients with unfavourable karyotype AML, while its role in CN AML cases is not yet established. **Methods.** We retrospectively analyzed 111 patients CN AML patients, considered at high risk according to initial clinical presentation or on the basis of poor response to induction therapy and candidate to allogeneic SCT. We evaluated the role of SCT in 51 patients who underwent transplant and its relationship with different know prognostic factors, including PGP expression at diagnosis. We also compared the survival in the transplanted patients with the 60 high-risk CN AML patients who did not received HSCT. **Results.** SCT was performed at a median of 7 months (range: 2-99) from diagnosis. Twenty-eight patients (55%) had a sibling donor, 25 an unrelated donor while 7 were cases of mismatching. Conditioning regimen was myeloablative in 40 cases (78%), non-myeloablative in 11. Acute GvHD developed in 17 patients (33%), mostly in MUD recipients. Chronic GvHD occurred in 14 of 41 evaluable patients (34%). One year TRM was 22% (11 cases). CR rate at engraftment was 90%. Sixteen of 21 (76%) patients transplanted with active disease obtained CR. Relapse occurred in 17 patients, with no difference according to PGP status at diagnosis. Allogeneic SCT conferred a significant advantage in survival. Median OS was 37 months in the transplanted patients, 12 months in the non-transplanted ones (p=0.007). The advantage of transplantation was evident both in the PGP+ and in the PGP- cases. Only HSCT was able to overcome the negative impact of PGP, with an identical OS in PGP-positive and PGP-negative transplanted patients. **Discussion.** Allogeneic SCT is an effective therapy in CN AML, especially in cases with high risk, such as over-expression of PGP. Transplant overcomes the impact of PGP on survival and may be pursued in patients who display this high risk feature at diagnosis.

**PO-107**

**Efficacy of allogeneic stem cell transplantation for advanced chemosensitive Hodgkin disease: The Bologna experience**


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**Introduction.** We have evaluated the outcome of allogeneic stem cell transplantation in 26 patients with advanced Hodgkin’s Lymphoma, refractory to multiple conventional treatments, including autologous stem cell transplantation. **Patients and Methods.** Median age was 31 years (16–47). Disease status at transplant was primary refractory or refractory relapsed disease in 16 patients (chemo-resistance patients) and sensitive relapse in 10 patients (chemo-sensitive population), as defined based on the attainment of at least a partial response following the last cycle of chemotherapy. The cell source was peripheral blood in 13 patients and bone marrow in 12 patients. Thirteen patients received transplant from a HLA identical sibling and 12 patients from a full matched unrelated donor. Cord blood was used only for one patient. Conditioning regimens were myeloablative in 6 patients (Busulfan plus Cyclophosphamide in 4 patients, and Busulfan plus Melphalan and Busulfan plus Fluorouracil in one patient each), and reduced intensity (RIC) (Thiotepa, Melphalan and Cyclophosphamide ± ATG Fresenius) for the others. **Results.** all
patients engrafted on median day 13 for neutrophils (ANC > 500/µL) and on day 14 for platelets (to > 50000/µL). Day 90 chimerism was consistently 100% donor in 23 evaluable patients and remained stable in all but one patient. Acute graft-versus-host-disease (GVHD) grade II-IV was diagnosed in 3/26 patients. Chronic GVHD was diagnosed in 4/26 patients. After a median follow-up of 578 days 15/26 patients are still alive but only 7 patients are in complete remission (all with chemosensitive disease at transplant). 7/11 patient died for relapsed/progressive disease whereas 5/11 patients died for transplant complication. 16/26 patients relapsed. 6 patients received DLI that caused GVHD in 4/6 but no durable response. The two-year OS (53±11% in chemoresistant vs 83±22% in chemosensitive, p=0.03) and EFS (0% in chemoresistant vs 52±23% in chemosensitive, p=0.0004) were significantly better in patients with chemosensitive disease. OS and EFS were not affected by the type of conditioning regimen, as well as by other transplant related factors. Conclusions: allogeneic HSC is potentially effective in patients with advanced Hodgkin Disease, provided the disease is still sensitive to chemotherapy. Results are disappointing in patients with chemoresistant disease.

PO-108
COMMUNICATION BIAS DURING THE DECISION MAKING PROCESS FOR UNRELATED BONE MARROW TRANSPLANTATION IN ADULT THALASSEMIA PATIENTS
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Introduction. This study combines the results of unrelated BMT performed in 34 adult class 3 thalassemia patients with investigation of communication factors between patients and physicians to assess whether communication bias, heuristics and distorted processes of remembering could affect the clinical decision-making process and compromise informed consent. Methods. Twenty-five surviving patients and 12 physicians were enrolled. A seven-item Likert-type scale was used to evaluate the following parameters in patients: the perception of mortality risk; the perception of GVHD risk; the perception of severe GVHD as a life-threatening condition; how much previous information had influenced the choice of BMT (to detect availability heuristics); patient’s motivation to undergo BMT (to detect anchoring heuristics); the numeric value of mortality risk considered acceptable and the value remembered by patients. Similar items were posed to the physicians. The last item on the questionnaire was used to detect the eventual presence of information framing effects. Results. The mean value of mortality risk perceived by the patients was significantly lower than the one communicated by the physicians (3.6 vs 4.9, p=0.001). The mean percentage of mortality risk communicated by the physicians was 80%, while the percentage recalled by the patients was significantly lower (20%, p=0.005). The mortality risk acceptable to the patients was significantly higher than the mortality risk acceptable to the physicians (29% vs 19%, p=0.005). The mean value of GVHD risk perceived by the patients was significantly lower than the value communicated (3.6 vs 5.3, p=0.001) and the perception of severe GVHD as a life-threatening condition was lower than among physicians (4.1 vs 5.4, p=0.006). Patients were strongly motivated to undergo BMT before informed consent and physicians were strongly conditioned by the hope that patients had already accepted to undergo BMT. Discussion. In conditions of uncertainty or risk, several judgment errors can be made in the course of communication between patients and physicians. This is because people tend to take shortcuts in reasoning, often driven by heuristics. Our results reveal a clear difference between the information supplied by the physicians and the perception of BMT risks among patients. Therefore, it is essential for physicians to improve communication strategies in order to make sure that the information conveyed is correctly understood by the patients. In this context, understanding, empathy and warmth can be powerful tools.

PO-109
GENOME-WIDE GENE EXPRESSION PROFILING COULD DISCRIMINATE PATIENTS, UNDERWENT ALLOGENEIC HEMOPHILIC STEM CELL TRANSPLANTATION (HSCT), WITH AND WITHOUT ACUTE GRAFT-VERSUS HOST DISEASE (aGVHD)
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Rationale. Modern approaches to predict the occurrence of aGVHD are needed. Genome-wide gene expression profiling might offer a new powerful tool to identify molecular onset of aGVHD following allogeneic HSCT. Herein, we report preliminary results from our longitudinal prospective study comparing expression changes of a gene pattern associated with acute inflammation in patients with (+) and without (-) aGVHD. Patients and Method. Eleven patients, undergone HSCT in different trials, were included in this study. WBC-RNA serial samples were collected between 10-90 days after SCT. All pts were transplanted with HLA identical related (n=7) and unrelated (n=4) donors. GVHD prophylaxis was performed with cyclosporin A plus methotrexate or mycophenolate mofetil. Seven patients did not experience aGVHD and 4 patients had grade II-III aGVHD, confirmed by cutaneous or intestinal biopsy. In this cases, molecular profiling before and after initiation of steroid therapy was investigated. TaqMan® Low Density Array was used to perform relative quantification of targets based on comparative CTdd CT method on Applied Biosystems 7900HT. Gene panel and functional classes are listed in Table 1, example of image plot in Figure 1. Results. We identified several genes that were up or down-regulated in aGVHD versus aGVHD-patients compared to their normal control. Remarkable changes in gene expression pattern were observed in aGVHD+ group. Signal transducer, activator of transcription, interleukin regulatory-inducer, immune-mediator genes (NFKB2, STAT-6, IRF8, MMP9) were significantly up-expressed as compared with normal controls or aGVHD-. Notably, CD52 m-RNA, a novel co-stimulatory molecule for induction of CD4+ regulatory T cells, and Fox-p3 levels were higher than controls. In addition, according to previous report, we observed CD83 up-regulation associated with IL-12A, IL-12B, IL-18 m-RNA over-expression. During the study period, sequential analysis of gene expression profiling showed no considerable changes in patients without aGVHD. As compared with their baseline values. Conclusions. Comparing normal and aGVHD+ group, significant gene expression changes could be identified. We believe that our array system may be used in future to identify transplant recipients not at risk for developing aGVHD. These results are encouraging for the establishment of a diagnostic tool useful to preemptive therapy of aGVHD.

Figure 1. Differential expression immune gene profiling detected by TaqMan® Low Density Array on a micro fluidic card
DECREASED TRANSPLANT MORTALITY IN PATIENTS WITH EARLY LEUKEMIA UNDERGOING AN UNRELATED DONOR TRANSPLANT


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Background. Unrelated donor (UD) transplants are being performed in increasing numbers worldwide. One of the major problems remains transplant related mortality (TRM). Aim of the study. To analyze the outcome of 300 consecutive UD transplants performed in Genova. Patients. Patients were transplanted in our Unit between 1989 and 2006. The diagnosis was aplastic anaemia (n=11), acute leukaemia (n=95), chronic myeloid leukaemia (n=118), lymphoma (n=19), myelodysplastic syndrome (n=34), other (n=23). Patients were stratified for year of transplant: 1989-98 (n=96), 99-2002 (n=108), 2002-06 (n=96). First remission patients (CR1) were 46%, 20%, 40% in the 3 eras (p=0.01). The median age was 34: the proportion of patients over 34 was 36%, 45%, 64% in the 3 eras (p=0.0005). Also the diagnosis was significantly different: CML represented 74%, 38%, 6% in the 3 eras (p=0.0001). Results. TRM in the three eras was 9%, 24%, 28% (p=0.04). The 3 year transplantation survival was 74%, 67%, 68% (p=0.02). The incidence of cGVHD was reduced in CR1 patients from 31% to 0% (p=0.004) and in advanced patients from 21% to 5% (p=0.04). Deaths due to infections were unchanged. In conclusion Despite significantly older age, TRM has been significantly reduced, mostly because of better GVHD prophylaxis. The benefit has been seen in early disease patients. Patients with advanced disease continue to have more problems.

DEVELOPING A NEW GRADING SYSTEM OF OCULAR SURFACE DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)


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Background and study objective. Ocular surface disease (OSD) resembling the dry eye syndrome is a common chronic GVHD-associated late complication of allogeneic HSCT and a cause of significant morbidity. Diagnosis of OSD is usually based on the measurement of tear production through the Schirmer test (ST); however, this test has low sensitivity and does not allow patient stratification. In this study we develop a more detailed grading system for OSD following allogeneic HSCT. Patients and Methods. Starting on april 2004, we enrolled 30 consecutive patients complaining of OSD symptoms (dryness, burning, hypersensitivities, pain or photophobia) after allogeneic HSCT. All enrolled patients underwent analysis of ocular surface function based on the following parameters: tear production (based on ST, scored positive if <5 in at least one eye); tear composition (scored positive based on either a positive fluorescing test or on a tear break-up-time of ≤5 seconds); cornal epithelial damage (scored positive based on slit lamp examination and by staining the cornea with fluorescein when possible); conjunctival inflammation (scored positive based on a conjunctival smear cytology score of ≥25). Each patient received a score (0 through 4) based on the number of positive parameters. Results. Patients were enrolled after a median follow up of 397 days (63-373). Two patients had a score of 0 (no OSD) and were therefore excluded from further analysis. Of the remaining 28 patients, 4 had a score of 1; 5 had a score of 2 (including 1 patient with a positive ST); for purposes of analysis, these two cohorts were grouped together as low risk patients; 7 had a score of 3 (including 3 with a positive ST (intermediate risk)); and 12 had a score of 4 (all with a positive ST) (high risk). Patient and transplant characteristics were similar in the three groups, with the exception of myeloablative conditioning, that was more common in the intermediate and high risk group (7/7 and 10/12, respectively) than in the low risk group (4/9). Among low risk patients 5/9 had cGVHD (3 extensive), whereas 5/7 had cGVHD in intermediate risk patients (4 extensive) and 12/12 in high risk patients (1 extensive). After a median follow up of 499 days following enrollment (range 0-1057, including two patients who did not receive follow up visits yet), the number of experimental treatments (including topical cyclosporine, autologus serum, plugs or protective lenses) that had been administered was 1 in the low risk group, 4 (all in 1 patient) in the intermediate risk and 14 (in 6 patients) in the high risk group. At last follow up, 5/9 patients in the low risk group had cGVHD (2 extensive), including 5 persistent patients with persistent OSD; 3/6 in the intermediate risk group had cGVHD (3 extensive), including 4 persistent OSD; and 11/11 in the high risk group had cGVHD (7 extensive), including 9 persistent OSD. Conclusions. Patients with a score ≥3 based on our OSD scoring system have a higher risk of persistent OSD requiring experimental treatment in association with refractory extensive cGVHD. The development of a more detailed OSD scoring system may allow better patients stratification and help monitor responses in clinical trials.

HUMAN LEUKOCYTE ANTIGEN-G 14-BASEPAIR POLYMORPHISM, A NEW PARAMETER TO PREDICT GRAFT VERSUS HOST DISEASE IN BONE MARROW TRANSPLANTATION


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HLA-G allelic variants characterized by a 14bp deletion-insertion polymorphism located in the 3’UT region of the HLA-G gene may determine an increment of molecular expression. We investigated the influence of the 14-bp deletion/insertion polymorphism on the outcome of allogeneic hematopoietic stem cell transplantation (HSCT) in 55 patients affected by thalassemia major. The donor/recipient pairs were completely identical at molecular level for HLA class I and II, HLA-G and HLA-G 14 bp genotypes. The conditioning regimen and GVHD prophylaxis were the same. Out of 53 transplanted patients, 39 are alive and well (disease free survival 73.6%), 8 rejected and 6 died. Sixteen patients (16/53-30.2%) developed grade II-IV acute GVHD. Patients that were homozygous for the 14bp deletion had a higher risk of developing cGVHD compared to patients homozygous or heterozygous for the 14bp insertion (+14bp/-14bp vs +14bp/+14bp: RR = 15.0; 95% CI 1.59-141.24; p=0.008). The presence of the 14bp insertion generates forms of mRNA that are more stable than others and thus promotes the expression of HLA-G molecules and immune tolerance. These results suggest that the 14bp polymorphism represents an important predictive factor for aGVHD occurrence after BMT. The evaluation of the 14bp polymorphism of the HLA-G gene in donor/recipient pairs should make it possible to better assess the risk for aGVHD and to adjust immunosuppressive therapy accordingly.

REDUCED TOXICITY PREPARATIVE REGIMEN WITH TRESOLSULFAN, THIOPETA, FLUDARABINE AND ATG IN UNRELATED ALLOGENEIC STEM CELL TRANSPLANTATION FOR THALASSEMIA PATIENTS


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Introduction. Allogeneic stem cell transplantation (aSCT) remains the only cure available for thalassemia patients. One of the most important problems in aSCT for thalassemia is the toxicity caused by high dose chemotherapy used for immunosuppression and eradication of the dis-
GVHD prophylaxis. To evaluate the possibility of reducing the transplant-related toxicity in thalassemic patients, we investigated a new conditioning regimen based on Treosulfan, Thiopeta, Fludarabine and ATG in thalassemic patients transplanted from unrelated donors. Sixteen thalassemic patients (7 males and 9 females, median age 18 years) underwent SCT from an unrelated donor selected by high-resolution HLA molecular typing. The conditioning regimen consisted of Treosulfan (14 mg/m² i.v. daily for 3 days) plus Thiopeta (8 mg/kg given in one day), Fludarabine (40 mg/m² i.v. daily for 4 days) and ATG (rabbit ATG Frese- nius, 10 mg/day), given for 3 days (days -5, -4, -3). Cyclosporine-A and short-term Methotrexate were used for graft-versus-host disease (GVHD) prophylaxis. Results. Fifteen patients (94%) are alive and transfusion-independent after a median follow up of 8 months (range 4-17); one patient rejected the transplant returning to the pre-transplant state. Four patients (25%) developed grade II-IV acute GVHD and 2 (15%) developed chronic GVHD. The treatment related mortality was 0%. Discussion. Our preliminary findings show that the experimental protocol was well tolerated by all patients. It is therefore possible to recommend its use in SCT, particularly in adult risk class 3 thalassemia patients.

PO-114
ROLE OF GVHD ON TRM AND RELAPSE AFTER ALLOGENEIC SIBLING MYELOABLATIVE PERIPHERAL STEM CELL TRANSPLANTATION
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Even if it’s largely recognized that PBSC transplants are associated with higher cGVHD incidence, the true benefit of cGVHD on transplant outcome (relapse and TRM) is still under debate. Here we report on a large series of 104 consecutive PBSC myeloablative allogeneic sibling transplants for haematological malignancies, with an homogeneous GVHD prophylaxis with CsA and methotrexate. Since the current classification schemes are not able to describe and separate all forms of cGVHD into moderate (less than 4 organs involved and/or Karnofsky index equal/more than 70%) or severe. With regard to transplant risk, patients were defined at standard risk if the diagnosis was acute leukaemia (AL) in first remission, chronic myeloid leukaemia in first chronic phase and chemosensitive multiple myeloma. All the remaining pts were considered as high risk. The overall incidence of cGVHD was 62.5%; of such cases 61.5% developed the extensive type. Median lines of therapy were 1 for limited, 2 for extensive-moderate and 4 for severe cGVHD; however, failure rate has been 78% and the probability of immunosuppression discontinuation has been less than 30% at 5 yrs for extensive cGVHD. Survival was affected by cGVHD (5ys OS has been 92% for limited cGVHD, 76% for cGVHD extensive-moderate, 70% for no cGVHD, 53% for extensive severe). TRM probability for the entire series was 25.7%, resulting lower, although not statistically, in the standard risk group (19% vs 27%, p=0.106). TRM was 50% at 5 yrs in pts with extensive-severe cGVHD whereas it was lower than 10% in all the remaining patients. On the contrary, the actuarial relapse probability was significantly influenced by the phase of the disease (14.5% at 3 years for standard risk vs 50.5% for high risk, p=0.0029) and no significant differences were found among pts with or without GVHD. We conclude that severe cGVHD negatively affects outcome after PBSC transplantation because it has more impact on TRM than on relapse and additional GVHD prophylaxis is needed.

PO-115
LONG TERM EVALUATION OF RELAPSE AFTER MYELOABLATIVE SIBLING TRANSPLANT FOR CHRONIC MYELOID LEUKEMIA
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Late relapses after allotransplantation are anecdotally reported, but analyses including very long observation times are unfrequent. Here we report on hematological relapse in a series of 79 CML patients who underwent allogeneic unmanipulated myeloablative transplant from HLA identical sibling at a single institution between 1983 and 1994 with a median follow up of 14.5 years. Median age was 36 years; 56 were in 1st CP, 17 in AP and 6 in BP. Preparative regimens were TBI based (10 Gy, single dose, low dose rate from a Co60 source) in 35 and Busulfan based (oral formulation, untargeted, 16 mg/kg total dose) in 44. GVHD prophylaxis consisted of CsA (28) or CsA+MTX (51). Bone marrow was the source of stem cells in all cases. Relapse occurred in 11 patients, resulting in 17.8% probability at 10 years. It was strongly correlated with phase at transplant (8.5% for 1st CP, 32.5% for AP and 60% for BP). Median times to relapse were 80 months for 1st CP, 52 for AP and 65 months for BP. Only two very late relapses occurred, at 135 (AP) and 137(1st CP) months. Of the 11 relapsed patients, 5 had had grade I acute GVHD, 9/11 did not have cGVHD and 2/11 had de-novo cGVHD. Out of 26 patient who developed cGVHD 2 relapsed, whereas 9/37 without cGVHD relapsed (p=0.08); 7 patients relapsed in advanced phase and died 9/3 of those transplanted in BP. 2/4 of those in AP and 2/4 of those in 1st CP. The 4 surviving patients, on the contrary, relapsed in CP and have achieved a II CR after therapy with IFN (1) or Glivec (3). These findings indicate that relapses are very rare, when the transplant is performed in 1st CP with a myeloablative regimen, even after a very prolonged observation. Nonetheless, very late relapses may occur.

PO-116
LONG-TERM RESULTS OF MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA
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In recent years, the use of allogeneic stem cell transplantation (HSCT) with myeloablative treatments for multiple myeloma (MM) has progressively decreased in favour of reduced-intensity regimens aimed at lowering TRM while maintaining a GVM effect. In the present study we retrospectively analyzed 45 patients (24 M, 21 F; median age = 45 yrs) who received myeloablative HSCT from 1995 to 2006 and were followed for a median of 4.5 years (0.08-10.9 years). Twenty-two patients received the graft from an HLA-identical sibling donor and 23 from a matched unrelated donor; median time from diagnosis to HSCT was 1.1 years (range 0.5-8.3). Median number of previous lines of therapy, including either single (31.1%) or double autologous SCT (13.4%), was 2 (range 1-4). Twenty-five patients (55.5%) had chemosensitive disease at transplantation, whereas the remaining 44.5% were refractory or progressive. A TBI-based regimen including Cy (120 mg/kg) and MEL (120 mg/m²) was used in 80% of the patients, while BU-Cy (16 mg/kg and 120 mg/kg, respectively) was administered in the remaining cases. Stem cell source was peripheral blood in 91% of cases. GVHD prophylaxis consisted of CSA+MTX in all the patients. Overall TRM was 22.5%. The overall rate of relapse or progression was 45%; median TTP was 6.9 years. In a multivariate analysis, the single most important and independent variable significantly extending OS, EFS and RFS was attainment of CR after HSCT (p<0.001, p=0.038, p<0.001, respectively). Chemosensitive disease at transplant (p=0.063), achievement of CR (p=0.056) and female sex (p=0.04) were significant predictors of prolonged duration of response. In conclusion, myeloablative HSCT does still represent a suitable therapeutic option for MM, mainly for younger patients with responsive disease prior to transplant. Benefits offered by this procedure in terms of prolonged duration of response and OS need to be balanced against the risk of TRM and chronic GVHD. Routine application of cytogenetics and molecular cytogenetics should aid a more accurate identification of those patients who are more likely to benefit from myeloablative HSCT rather than nonmyeloablative allogeneic transplantation.
Non transferrin-bound iron (NTBI) is a chelatable, low weight plasma iron fraction. Previous studies have suggested that during myeloablative chemotherapy there is a transient appearance of NTBI due to a temporary reduction in iron removal from transferrin by the erythron and to cell breakdown. This free form of iron can mediate the production of reactive oxygen species and could cause organ toxicity. The aim of our study was to evaluate the NTBI kinetic in iron overloaded thalassemic and non iron overloaded non thalassemic patients during cytotoxic therapy for bone marrow transplantation and verify its relationship to clinically detectable toxicity. Eighteen patients affected by β-thalassemia major (8 female, 10 men) median age 8.8 (4-16 years) and nine affected by different kinds of haematologic malignancy (6 female, 3 men) median age 27.4 (10-44 years) were recruited for this study. The median ferritin in thalassemic patients was 2290 µg/mL (range 759-6360 µg/mL), while in non thalassemic patients was 949 (range 27-3916 µg/mL). The median hepatic iron concentration (HIC) in thalassemic patients was 15.4 mg/dry weight (range 4.09-40.00), in non thalassemic patients it wasn’t sampled but normal value is lower than 1.6 mg/dry weight. Patients were treated with high doses Busulfan (14-28 (Figure 1). group compared to that of non thalassemic patients at days –9, 0, 14 and during therapy (Figure 1). The median difference in iron burden. No correlation to transplant related toxicity (grade II). None of the patients presented acute GVHD major than grade II and 3 patients experienced chronic GVHD. Among non thalassemic patients: 2 developed liver toxicity (grade I), 3 had gastro enteric toxicity (1 grade II, 2 grade I), one had mucosal toxicity (grade II), none developed cardiac and cutaneous toxicity. 4 presented acute graft versus host disease (GVHD) major than grade II and 3 patients experienced chronic GVHD. Among non thalassemic patients: 4 developed liver toxicity (3 grade I, 1 grade II), 4 had gastro enteric toxicity (3 grade II, 1 grade I), 4 had mucosal toxicity (2 grade I, 2 grade II), one developed bladder toxicity (grade II). None of the patients presented acute GVHD major than grade II and 4 patients experienced chronic GVHD. This study indicates that the increase of NTBI in iron overload and in non iron overload patients, during the peri transplant phase, wasn’t relevant in spite of the hight difference in iron burden. No correlation to transplant related toxicity and transplant outcome was observed.
Patients and Methods. Transplantation (HSCT) in elderly patients (pts). In the present analysis cell transplantation. Variable intra and inter systemic drug exposure as measured by area under curve (AUC), can occur following oral administration and may be influenced by age, body weight, absorption variability and, particularly in infants, by difficulties to assumption. Aims. To evaluate the clinical outcomes, the toxicity and the compliance to iv BUS in children undergoing to pre-transplant conditioning regimen. Methods. Thirty-four patients (pts), median age 37 (17-77) m, body weight 6-69 kg, affected by AML (7; 6 in I CR, 1 in II CR), ALL (7; 2 in I CR, 5 in II CR, 2 in RR), NB (10; 5 in I CR, 7 in I PR), ICH (2), Ewing sarcoma (8; 2 in I CR, 4 in I PR), secondary graft failure. Moreover allows to avoid assumption difficulties, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells have been reported to modulate both in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells did not modify the CD25 expression. In one no contact experiment, the inhibition index was half of that conditioned medium from supernatant of mesenchymal cells cultures. In 

PO-119
CLINICAL RESULTS OF AUTOLOGOUS OR ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWING A NEW INTRAVENOUS BUSULFAN DOSING AS PART OF CONDITIONING REGIMEN IN CHILDREN: EARLY CLINICAL OUTCOMES TOXICITY AND COMPLIANCE
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Background. High dose BUS is an important component of pre-transplant conditioning regimen in children with advanced haematological malignancies or solid tumour undergoing allogeneic or autologous stem cell transplantation. Variable intra and inter systemic drug exposure as measured by area under curve (AUC), can occur following oral administration and may be influenced by age, body weight, absorption variability and, particularly in infants, by difficulties to assumption. Aims. To evaluate the clinical outcomes, the toxicity and the compliance to iv BUS in children undergoing to pre-transplant conditioning regimen. Methods. Thirty-four patients (pts), median age 37 (17-77) m, body weight 6-69 kg, affected by AML (7; 6 in I CR, 1 in II CR), ALL (7; 2 in I CR, 5 in II CR, 2 in RR), NB (10; 5 in I CR, 7 in I PR), ICH (2), Ewing sarcoma (8; 2 in I CR, 4 in I PR), secondary graft failure. Moreover allows to avoid assumption difficulties, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells did not modify the CD25 expression. In one no contact experiment, the inhibition index was half of that conditioned medium from supernatant of mesenchymal cells cultures. In

PO-121
MESENCHYMAL CELLS INHIBIT IL2 AND ZOLEDRONATE-INDUCED γδ T-LYMPHOCYTES
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Gamma/delta AT-cells have a central role in the communication with many different cell subpopulations and realize a link between specific and unspecific immunity. Their role in the control of cancer has been previously reported and it has been shown to be implicated in the graft-versus-host disease. In this study, γδ T-cells were significantly increased when IL-2 10 U/mL and zolendronate 0.1 uM were added; the absolute number of viable cells and CD8 cells was not significantly increased. Expanded γδ T-cells were CD4, CD8, CD69, CD25, Vδ2TCR, F9TCR. The memory cells mean percentage was significantly higher than the effector cells. Molecular monitoring of TCR repertoire confirmed that the expanded γδ T-cells were F9/VδTαδ2. T- and T-cell spectraprobe showed a polyclonal pattern. IL-2-zoledronate-expand ed cells were able to induce an increased specific lysis of the myeloma RPMI 8226 cell line, compared to the lysis induced by control cells cultured without IL2 and zoledronate. Any significant specific lysis of HL 60 promyelocytic cell line was induced by the same cells. Moreover, the addition of mesenchymal cells to the PBMC cultured with IL2-zole dronate in a 1:1 ratio almost completely abolished the generation of gamma/delta T-cell. To evaluate the possible role of cytokines produced by mesenchymal cells, the half of culture medium was substituted with conditioned medium from supernatant of mesenchymal cells cultures. In these conditions, the anti-proliferative activity was lower (11% vs 82%). In one no contact experiment, the inhibition index was half of that detected in a parallel contact experiment (41%). Cytotoxic immumetric evaluation showed that mesenchymal cells did not modify the CD25 expression. Mesenchymal cells have been reported to modulate both in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells decrease the expression of CD4 activation markers, CD25, CD38 and CD69 on PHA-stimulated lymphocytes and their suppressive activity is partially independent from cell contact, with production of several inhibiting substances, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells decrease the expression of CD4 activation markers, CD25, CD38 and CD69 on PHA-stimulated lymphocytes and their suppressive activity is partially independent from cell contact, with production of several inhibiting substances, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells decrease the expression of CD4 activation markers, CD25, CD38 and CD69 on PHA-stimulated lymphocytes and their suppressive activity is partially independent from cell contact, with production of several inhibiting substances, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells decrease the expression of CD4 activation markers, CD25, CD38 and CD69 on PHA-stimulated lymphocytes and their suppressive activity is partially independent from cell contact, with production of several inhibiting substances, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system. Mesenchymal cells decrease the expression of CD4 activation markers, CD25, CD38 and CD69 on PHA-stimulated lymphocytes and their suppressive activity is partially independent from cell contact, with production of several inhibiting substances, such as nitric oxide and interleukins. These results could be particularly relevant in the context of dissociation between in vitro and in vivo the immune response and inhibit T-cell alloreactivity, independently from the major histocompatibility system.
cells a very interesting tool for clinical application in the prophylaxis of GVHD. Finally, the above reported data would suggest that the inhibiting activity of mesenchymal cells in multiple myeloma may be at least in part mediated by their inhibition on the proliferation of this sub class of T-lymphocytes.

**PO-122**

EXTRACORPOREAL PHOTOCHEMOTHERAPY IN TREATMENT OF ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE

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Photopheresis (extracorporeal photochemotherapy, ECP) characterized by exposure of peripheral blood mononuclear cells to the photosensitizing agent 8-methoxypsoralen and UV-A radiation, has been shown to be effective in treatment of selected oncological and autoimmune diseases. Additionally, recent reports indicate that this therapy is promising also in patients who develop Graft versus Host Disease (GvHD) after allogeneic hematopoietic stem cell transplantation (HSCT) for hematological malignancies. We present 19 patients (with a median age of 48 years; 13 developed an acute GvHD (aGvHD), 11 grade II, 1 grade III and 1 grade IV) and 6 a chronic GvHD (cGvHD, 3 limited and 3 extensive) after (HSCT) and were referred to ECP. All patients were refractory or poor responders to steroid therapy. Seventeen patients received a stem cell grafting from sibling donor and 2 from unrelated donors while 8 were treated with an ablative conditioning and 11 with a nonmyeloablative regimen. The stem cell were collected from peripheral blood in 18 cases and from bone marrow in 1 case. The majority of patients had skin and liver involvement (only 3 patients with aGvHD had gastrointestinal involvement). The ECP procedure was performed using the UVAR XTS photopheresis system (Therakos Inc., West Chester, PA, USA). Patients were treated on 2 consecutive days (one cycle) every week for two months, every two weeks for the following two months and then ECP was tapered on an individual basis. The median number of treatments was 21 and no adverse events were observed. Of the 13 aGvHD patients, 5 (45%) achieved a complete response (CR) of GvHD manifestations, 2 (15%) a partial remission (PR) and 4 no response. In the 6 cGvHD patients, 3 obtained a CR and the other 3 a PR. The immunosuppressive therapy was discontinued in 32% of patients (all cases with grade II aGvHD or limited cGvHD) and reduced in 57%. Our findings suggest that ECP is an effective adjunct therapy for both acute and chronic GvHD with cutaneous and liver involvement. In addition, this beneficial effect is obtained without the complications typically encountered with immunosuppressive regimens used to control GvHD. However, in patients with aGvHD grade IV or extensive cGvHD other therapeutic options are warranted. Randomized studies on a larger number of patients are needed to confirm the efficacy of ECP in GvHD and its role in the treatment of this complications after HSCT.

**PO-123**

ANALYSIS OF RELAPSE AFTER ALLOGENEIC MYELOABLATIVE STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: PROGNOSTIC FACTORS AND PATTERNS OF RELAPSE


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Allogeneic SCT can affect a high rate of response in Multiple Myeloma (MM). However, relapse and progression still remain a major concern for the final outcome. From 1983 to 2004, 97 MM patients received a sibling myeloablative allogeneic SCT. Median age was 42 years, 49 were chemoresistant, 59 received bone marrow and 38 PB as source of stem cells. Conditioning regimens were total body irradiation, cyclophosphamide and melphalan in 65 patients; busulfan and cyclophosphamide were used in the remaining pts. Graft-versus-host disease (GVHD) prophylaxis consisted of Cyclosporin-A (Cs-A) and short term MTX for T-depleted transplants (68%), T depletion (30%) was made using CamPATH-1H or CD34 positive selection. 2 haploidentical transplants didn’t received any prophylaxis for GVHD. With a median follow-up of 72 months, 41 patients relapsed (19) or progressed (22), whereas 11 patients experienced a remission of 10 years or more, including the longest at 25 years. Overall, the median time to relapse/progression was 52.6 months; the 5-year probability of post-transplantation relapse/progression was 47% for patients in CR or VGPR at transplant as compared with 75% for the remaining patients. Median survival time from disease recurrence was 39 months for patients in CR or VGPR at transplant and 15 for all the remaining patients. In a multivariate analysis, an early pretransplant stage (I vs III) and response after SCT (CR and VGPR) resulted independent prognostic factors for relapse/progression. We defined three patterns of relapse or progression localized (single osteolytic lesion or extramedullary lesion), systemic (serological, or urinary or bone marrow involvement) and mixed (localized and systemic). We recorded 2 localized, 18 systemic and 21 mixed recurrences of MM. For recurrence of the mixed type time elapsing from the first appearance of localized lesion and the systemic recurrence was between 7 and 18 months. No relationship was found between the patterns of relapse, pre-transplant and transplant-related variables, as well as with post-transplant duration of survival. Although recurrence of MM still remains a major issue of allogeneic SCT, long term control of the disease is recognized in a sizeable proportion of patients. Post-transplantation use of novel agents is a promising treatment option to reduce the risk of relapse/progression and/or to prolong the duration of remission.

**PO-124**

IRON OVERLOAD AFTER T-CELL DEPLETED HSCT FROM EITHER MATCHED OR MISMATCHED DONORS


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Methods. Incidence and treatments of the iron overload secondary to hematopoietic stem cell transplantation (HSCT) was retrospectively assessed in recipients of allogeneic T-cell depleted transplants. Between March 1990 and November 2005, 162 patients have been transplanted from either HLA-matched (n=95) or HLA-mismatched donors (n=67). The median follow-up was 95 months (range 11-203). All long-term survivals were screened for the serum ferritin levels. Results. Hyperferritemia was documented in 52 of the 162 patients (32%) at a median of 28 months after transplantation (range 6-100). The ferritin levels resulted >2 times higher than the normal in 38 patients, were only slightly increased in the other 14. Ferritin levels were significantly higher in patients transplanted for MDS or secondary leukemia than in those with de novo AML (p=0,019), ALL (p=0,013) or CML (p=0,066). The following co-morbidities were documented: Hepatitis virus B or C in 5/52 (9,6%), chronic GVHD in 2/52 (3,8%), hemolytic anemia in 12/52 (23%). 13/14 patients whose ferritin was <2 times the normal values have not received any specific treatments and normalized their ferritin levels; one patient successfully responded to administration of erythropoietin which was used to treat the concomitant chronic renal failure. The 38 patients with ferritin levels >2 times the normal have received the following therapies: a) phlebotomy (n=5) for a median of 8 months (range 6-12); 4 normalized the ferritin levels, 1 was refractory; b) desferoxamine (n=9) medially for 12 months (range 2-20): 3 achieved a complete remission, 2 were refractory and 4 are still on therapy; c) phlebotomy combined with recombinant erythropoietin (n=15) for a median of 5, 6 months (range 3-12): 11 normalized serum ferritin levels, 1 was refractory and 1 is still on therapy; d) erythropoietin was the only treatment in one patient who is still on therapy; e) phlebotomy has been recently started in the last patient. Ten patients refused any treatment. Conclusions. Iron overload was documented in 32% of patients undergoing HSCT in our center. MDS and secondary leukemia were the most significant risk factors for iron overload. Unlike the thalassemic patients there are no guidelines for leukemic patients undergoing HSCT. Nevertheless, combining phlebotomy with erythropoietin safely normalized serum ferritin levels and contributed to improve quality of life.
PO-125

HIGH DOSE ACYCLOVIR FOR CYTOMEGALOVIRUS INFECTION PROPHYLAXIS AFTER ALLOGENIC STEM CELL TRANSPLANTATION

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Acyclovir (ACV) is the first effective antiviral agent for the prevention of cytomegalovirus (CMV) reactivation in stem cell transplanted patients. Herein, we compared two groups of patients receiving, iv ACV from day -7 to day +20 after transplant followed by low-dose oral ACV (200 mg x 4/day) (group A) or high-dose oral ACV (800 mg x 4/day) (group B) for a period of 6 months after transplant. ACA of the study was to evaluate differences in CMV infection (detected by pp65 antigenemia), CMV disease, other herpes virus diseases, adverse events, probability of survival and transplant-related mortality (TRM) between the two groups. We analyzed 116 patients managed between January 1999 and November 2006. The first 60 received low-dose oral ACV; from March 2004, we prospectively administered in 56 patients high-dose oral ACV. The two groups were comparable for age, sex, disease, phase at transplant, CMV serology combination between donor and recipient at transplant, HLA compatibility, conditioning regimens and supportive therapy. No differences were observed between the two groups considering the rate of engraftment and acute and chronic GVHD. The cumulative incidence of CMV infection was 70% in group A and 54% in group B. This difference was statistically significant (p=0.03). We observed CMV disease in 2 patients in group A and in 1 patient in group B. Herpes virus disease was not observed in both groups during the first 6 months after transplant. Observed adverse events that were possibly related to treatment were nausea and vomiting (55% group A vs 66% group B) and creatinine increase (45% group A vs 56% group B). The 1-yr cumulative incidence of TRM was 17% and 21% for groups A and B, respectively (p=ns). The 1-yr probability of survival was 78% in both groups. In conclusion, we showed the efficacy of ACV prophylaxis to control the incidence of CMV infection. In the future, a trial of low-dose oral ACV will be evaluated in comparison with the high-dose oral ACV.

PO-126

ORIENTATION PROGRAMME FOR NURSES ENTERING A BONE MARROW TRANSPLANT UNIT FOR THE FIRST TIME

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Introduction. This programme aims at integrating nurses with no previous experience in a Bone Marrow Transplant Unit into the new environment with its policies and roles. By the end of this orientation programme, the nurse becomes able to function independently and carry out the responsibilities assigned to her/him within the unit. The integration process is extremely important and requires the collaboration of the experienced and independent staff members who have to guide, step by step, the new colleague in all the activities of nursing practice. The experienced nurses, on the other hand, can find in this process, the opportunity to extend and update their own Knowledge. Objectives. To standardize the procedures, to decrease the possibility of errors and accidents, to reduce time for new nurses to become independent, to improve job quality. Methods. The orientation programme is based on the use of written guidelines about transplant unit procedures and on a real time new members’ support from experienced staff. Conclusions. Encouraging orientation and education of new nurses entering a Bone Marrow Unit is extremely important to improve the quality of all transplant unit activity and may also provide a great opportunity for experienced staff to enhance their professional skills.
Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Transplant Source</th>
<th>Relapse Site</th>
<th>Time from BMT (days)</th>
<th>GVHD</th>
<th>Subsequent Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>SIB/RIC</td>
<td>Kidney</td>
<td>60</td>
<td>y</td>
<td>Surgery + CHT</td>
</tr>
<tr>
<td>MM</td>
<td>SIB/RIC</td>
<td>Skin</td>
<td>43</td>
<td>n</td>
<td>CHT</td>
</tr>
<tr>
<td>MM</td>
<td>SIB/RIC</td>
<td>CNS</td>
<td>4</td>
<td>n</td>
<td>CHT</td>
</tr>
<tr>
<td>MM</td>
<td>SIB/ABL</td>
<td>Muscle, skin, kidney</td>
<td>8</td>
<td>y</td>
<td>RT + CHT</td>
</tr>
</tbody>
</table>

Conclusions. Our data confirm the relatively rare occurrence of isolated extramedullary relapses following allogeneic transplant. In our experience, patient outcome is poor in spite of subsequent treatments: 1 patient, affected by MM is alive with disease, 2 patients affected by AML died from progressive disease, the fourth MM patient is alive with smouldering disease. A multicentric study is warranted to analyze a larger series of patients in order to evaluate the real impact of GVHD on the extramedullary relapse occurrence.

PO-129

USE OF VALPROIC ACID (VPA) IN ALLOGENEIC HEMOPOIETIC STEM CELL (HSC) TRANSPLANTATION: A SAFETY AND FEASIBILITY STUDY


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Background. Local chromatin remodelling and changes in nucleosomal packaging of DNA are key steps in the regulation of gene expression affecting cell proliferation and differentiation. VPA, acting as an inhibitor of histone deacetylases, allows the access of transcription factors to DNA. Recently, several studies have shown that VPA affects normal HSC proliferation by up-regulating genes involved in stem cell cycling and self-renewing. Aims of the study. VPA has been safely used in neurology and, more recently, in haematological malignancies. The aim of this study was to evaluate clinical safety and feasibility of VPA administration in patients undergoing allogeneic transplant. The treatment started before transplant, as prophyaxis of neurological complications of conditioning regimens, and continued for one month after transplant to evaluate VPA effect on early HSCs engraftment. Material and Methods. From June 2006 to February 2007, 15 pts (9 M, 6 F) entered the study. Median age of patients (range 26-63). Five pts were affected by AML, 3 by ALL, 1 by NHL, 1 by HD, 1 by MM. Six pts underwent matched (5 BM, 1 PBSC) and 9 pts unrelated (6 CB, 3 PBSC) HSCT. No patient was affected by haepatic, renal or neurological impairment. Conditioning regimen was myeloablative in 10 pts and at reduced intensity in 5 pts. VPA was administered at initial dose of 200 mg/day (from day -10 to -7) and then the dose was increased at a monthly interval, up to 1000 mg/day at day -2. After HSC infusion the administration was continued at dose of 400 mg iv twice a day until recovery of suitable oral absorption and then pursed by orally formulation until day +30. Pts were daily monitored for VPA serological level, cyclosporine, haepatic and renal functional parameters. Serological VPA concentrations between 50 and 100 µg/ml administrated by patient weight (15-30 mg/kg) and subsequently modulated according to clinical tolerance.

PO-130

ESTROGEN-PROGESTIN REPLACEMENT THERAPY IN WOMEN AFTER STEM CELL TRANSPLANT


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Introduction. Transient or persistent premature ovarian failure (POF) is the most common complication reported after stem cell transplant (SCT), occurring in more than 90% of women. Methods. A group of women in their reproductive age (45 allo- and 89 auto-transplanted, aged 18 to 45 yrs, median 31) who had received myeloablative SCT for malignant hematopoietic disorders in our institution were evaluated for in a pilot study with estrogen-progestin-replacement therapy (EPT) 6-24 months after SCT. We chose to give them a cyclic sequential treatment with 2 mg estradiol daily associated with 10 mg dydrogesterone for 14 days per cycle. All the patients were followed up for at least 12 months. Results. In our cohort of 125 patients, 59% started EPT after SCT: 25 allo and 34 auto-transplanted and 46 auto-transplanted women; the median period of treatment was 35.8 months (range, 10-75). One third of women refused EPT, and in 15% EPT was contraindicated for presence of liver GVHD. The EPT treatment was stopped in 3 auto-transplanted patients who experienced recovery of menstrual cycles during EPT; other 2 women stopped the treatment when their disease relapsed. Only one woman was taken off EPT because of persistent headache. The other 54 women are still on treatment. This means that, in our experience, the use of the combined cyclic oral EPT was safe, well tolerated and associated with optimal compliance (98%). The incidence of irregular bleeding was very low (2%). During the first 1-4 months of EPT administration, 10% of treated women had mild regular bleeding, which increased thereafter. As for the effects of EPT in the autologous setting, such a treatment reduced all symptoms related to cycle disappearance, and improved bone mineral density (BMD). In the allogeneic setting, the treatment reduced all symptoms related to cycle disappearance but did not effect BMD. Estradiol plus dydrogesterone administration reduced vasomotor symptoms and those of urogenital atrophy within 2-4 months. EPT did not exacerbate GVHD in previously affected women and did not worsen any parameter of cutaneous or any other cGVHD form in women who started EPT having a mild chronic cGVHD. Conclusions. EPT was associated with a excellent compliance, due to very few adverse effects, and led to a dramatic improvement of vasomotor, genitonal and psychological symptoms related to estrogen deficiency.

PO-131

INFLUENCE OF CYTOKINE GENES SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) ON THE OUTCOME OF HEMATOPOIETIC ALLOGENEIC STEM CELL TRANSPLANTATION (HSCT)


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Polymorphisms in cytokine genes and their promoters have been shown to influence hematopoietic stem cell transplantation (HSCT) outcome. However published data is still controversial. Methods. To summarize published data we performed a standard search in Pubmed. Original papers published by peer-reviewed journals between 1998 and 2006 were selected (23 papers from 15 centres). We evaluated 64 consecutive patients (59 patients) affected by hematological malignancies transplanted from 1998 to 2006 with HLA identical sibling donors. Median age: 48 years. 36 myeloablative conditioning, 26 reduced intensity. Stem cells source: 31 bone marrow, 35 peripheral blood. In our experimental setting we performed a PCR-SSP to detect cytokine SNPs positions -592 (A/C), -819 (T/C) and -1082 (A/G) of Interleukin10 (IL10); position -308 (A/G) for Tumor Necrosis Factor alpha (TNFa); position -174 (G/C) for Interleukin 6 (IL6); position +874 intron 1 (T/A) for Interferon-gamma (IFNg); positions at codon 10 (T/C) and codon 25 (C/G) for Transform-
**PO-133**

**SUBSETS OF CD34 AND ENGRAFTMENT KINETICS IN ALLOGENIC PERIPHERAL STEM CELL TRANSPLANTATION IN AML PATIENTS**


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Engraftment kinetics in allogeneic peripheral blood stem cell transplantation (alloPBSCT) depend on the number and efficiency of the stem cells in the graft, the conditioning regimen and GVHD prophylaxis. Currently, stem cell evaluation is performed by counting CD34+ cells; however, CD34+ cells are a heterogeneous population including the early uncommitted hematopoietic cells as well as differentiated progenitors coming from another lineage; hence, defining the CD34+ subset most predictive of engraftment and its threshold value would be of the utmost importance. This study aimed to investigate which graft product subset of CD34+ cells might be the most predictive of early hematopoietic recovery following alloPBSCT. The relationships between the number of mature subsets of CD34+ cells (CD34+/CD33–, CD34+/CD38–, CD34+/CD133– and immature subsets of CD34+ cells: CD34+/CD38+, CD34+/CD133+, CD34+/CD133–) and early neutrophil and platelet engraftment were studied in a homogeneous series (for disease, pre-transplant chemotherapy, conditioning regimen GVHD prophylaxis) of 31 acute myeloid leukemia (AML) patients after alloPBSCT from HLA-identical siblings. All patients received the BU-CY regimen consisting of busulfan 4 mg/kg/day for 4 consecutive days followed by cyclophosphamide 60 mg/kg/day for 2 consecutive days; GVHD prophylaxis included cyclosporin and methotrexate. The CD34+ dose infused ranged from 2.9 to 8.8×10^6/Kg (median: 6.8); the percentage of immature CD34+ cells was 40% for CD34+/CD33–, 65% for CD34+/CD38–, 5% for CD34+/CD133– and 70% for CD34+/CD133+. This translates into a median dose of 1.8×10^5/Kg (range 0.3-5) for CD34+/CD33–, 2.8×10^5/Kg (range 0.1-6.2) for CD34+/CD38–, 0.3×10^5/Kg (range 0.05-2.3) for CD34+/CD133– and 3.2×10^5/Kg (range 0.6-7.5) for CD34+/CD133+. Median time to achieve engraftment of neutrophils and platelets was 13 (range 10-16) and 19 (range 13-19) days, respectively. In our experience the total CD34+/CD133– cell number was inversely correlated with the days required for recovery of 0.5×10^9/L neutrophils (r = -0.82, p = 0.02) and 20×10^9/L platelets (r = 0.80, p = 0.06); this correlation was better than the total CD34+ cells dose and neutrophil (r = 0.70, p = 0.05) and platelets engraftment (r = 0.56, p = 0.07). No correlation was found between the other CD34+ subsets neutrophil and platelets engraftment. We suggest that a high number of CD34+/CD133– peripheral blood stem cells may be associated with faster neutrophils and platelets recovery; these findings may help to predict the repopulating capacity of PBSCs in patients after allogeneic PBSCT, especially when a relatively low number of CD34+ cells is infused.

**PO-132**

**TREOSULFAN/FLUDARABINE AS PRE-TRANSPLANT CONDITIONING FOR PATIENTS WITH HIGH RISK HEMATOLOGIC MALIGNANCIES AND HIGH COMORBIDITY INDEX**


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Treosulfan (a new busulfan derivative alkylating agent) has demonstrated antitumoral and immunosuppressive activity in pre-allogeneic stem cell transplant setting (allog HSCT) in high risk hematologic patients. For this reason we started a Treosulfan based conditioning with the aim to reduce toxicity and Transplant Related Mortality (TRM) in patients with high malignancies and comorbidities, meeting criteria for standard conditioning regimens. Evaluations of Overall Survival (OS), Disease Free Survival (DFS), TRM and relapse were secondary objectives of the study. Since July 2005 to March 2007, 39 consecutive patients (20 males, 19 females) entered this study. Mean age was 45 years (range 17-65). Underlying diseases were: Acute Leukemias (ALL, 10 AML, 4 CML, 3 MM, 1 Histiocytic Sarcoma, 1 HD, 2 IMF). All patients were heavily pretreated; only 15/39 were in CR at the moment of the transplant; mean Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) was 2 according to the Seattle criteria. Conditioning consisted of Treosulfan 12-14 gr/m2 for 3 days in combination with Fludarabine 30 mg/m2 for 5 days. Cylosporine plus short MTX were used as GVHD prophylaxis; anti-Thymocyte globulin (Thymoglobulin) was used in patients receiving PBSCs and MUD transplants. Twenty-two (22) patients received HSCTs from HLA identical siblings and 17 with unrelated donors. Source of stem cells were: bone marrow in 14 patients and peripheral blood stem cells in 25 patients. Thirty-seven (37) patients (95%) engrafted; mean time to neutrophil >500×10^9/L was 16 days (range 10-26), to platelets >20000×10^9/L was 16 days (range 10-24). One (1) patient did not engraft (a sAML patient with previous thyrroid cancer and iodine 131 radiation); 1 patient was not evaluable for early death at day +9; Four (4) patients experienced Gastro-Intestinal (GI) toxicity (2 grade II, 2 grade III); 1 patient presented grade II liver toxicity and 5 grade I, 1 patient had grade I renal toxicity. Six (6) pts presented acute GVHD (4 grade I, 2 grade II). One patient had CrGVHD and it was limited. Twenty-one (21) patients were alive (54%); KM-probability OS curve at 19 months is 39% (CI 20-59); 100 days - OS is 77% (CI 64-91). Eighteen (18) patients (46%) are in complete remission with a median follow-up of 9 months (range 1-19 months). Eighteen (18) patients died (46%); 13 for recurrent disease, 4 for TRM, 1 for a Transient Ischemic Attack (TIA) 3 months after transplant. This preliminary study underlines that Treosulfan-Fludarabine association is an effective conditioning regimen even in high risk patients with advanced phase of disease. Engraftment is rapid, regular and sustained and post transplant toxicity is reduced. Longer follow up is necessary to evaluate the anti-tumor capacity of this regimen but disease status at transplant is the most important risk factor for outcome (OS 60% for patients in CR vs OS 28% for patients in relapse).

**PO-134**

**AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) FOLLOWED BY ALLOGRAFT WITH NON-MYELOABLATIVE CONDITIONING (TANDEM PROTOCOL) IN MULTIPLE MYELOMA PATIENTS: A SINGLE CENTER EXPERIENCE**


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Patients and Methods. Since January 2001 to April 2007 26 consecutive patients (pts) older than 50 years with newly diagnosed Multiple Myeloma (MM) (M 16, F 10; stage IIIA 28, IIIB 3) underwent HSCT autograft followed by an allograft with non-myeloablative conditioning (tandem protocol). The pts received HD Melphalan (200 mg/m2) followed by autologous PB-SCT. After a median of 90 days, the pts underwent RICT (Fludarabine + 2 Gy TBI). Acute GVHD prophylaxis consisted of MM and cyclosporine. Chimerism analysis was performed using STR-PCR and donor engraftment was evaluated at day +15,+30,+45,+60,+90 on unfractionated BM cells. All pts received a HLA identical donor mobilized PBSC and the graft contained a median of 5.3×10^9 (range 1.6-8×10^9) CD34+ cells/kg body weight. After RICT, on day +15, 4 (15%) pts showed a complete donor chimerism; on day +90, 24 (92%) showed a complete donor chimerism; two pts with mixed chimerism received a DLI on day +30 and one of these achieved full donor chimerism. Results.
Grade I-II and III acute GVHD occurred in 5 (19%) and 2 (7%) pts respectively. Five pts (19%) developed a mild and 5 (19%) an extensive chronic GVHD. Eleven pts (42%) achieved CR and they are in CCR at +72,+72,+65,+64,+61,+35,+30,+19,+15 and +15 months; 1 patient in CR died for cardiopathy. Five pts (20%) are in PR. Nine (35%) pts not in CR showed a progressive disease and 7 (27%) of them died. With a median follow-up of 30 months, 18 (70%) are alive. Conclusions. We demonstrated that survival after allogeneic transplantation is favourable: 62% of all pts achieved CR or PR. None patients died after RICT. Pan cytopenia after RICT was minimal and sustained allogeneic stem cell engraftment occurred in 90% of patients. A good correlation between GVHD, full chimerism and remission was found. All patients in CR developed acute/chronic GVHD and the presence of GVHD correlated with a lower relapse rate. In all patients the achievement of CR was gradual and a constant regression of the monoclonal band was observed.

### Table 1

<table>
<thead>
<tr>
<th>No blasts</th>
<th>Blast≤5%</th>
<th>Blast&gt;5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS CR1</td>
<td>39</td>
<td>30</td>
</tr>
</tbody>
</table>

The cumulative incidence of transplant related mortality (TRM) was 34%, 35% and 69% in the 3 groups of patients (p=0.0006) (Table 1). Relapse related death (RRD) was 5%, 10%, 20% (p=0.1). With a median follow-up of 1557, 2583 and 1384 days, the proportion of patients surviving at 10 years is respectively 57%, 44%, 6% (p<0.0001). There was no difference in survival between patients grafted from identical siblings or unrelated donors; age (<51 years) was not a significant predictor of survival. Conclusions. We confirm that long term survival can be achieved in hypoplastic MDS with no blasts at diagnosis, irrespective of donor type. MDS with blasts at diagnosis have similar outcome only if grafted in CR1, after induction chemotherapy. MDS patients diagnosed with blasts and who come to transplant without induction or beyond CR1 have a very poor outcome.

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**CHRONIC LYMPHOCYTIC LEUKEMIA AND LYMPHOPROLIFERATIVE SYNDROMES**

## PO-136

**ZAP-70 NEGATIVE B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA TRANSFORMED TO HODGKIN LYMPHOMA: A CASE REPORT**

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The transformation of B-cell Chronic Lymphocytic Leukemia (CLL) into more aggressive lymphoproliferative disorders in the form of Hodgkin lymphoma (HL) is a rare event portraying a very poor prognosis. From the analysis of CLL biologic features predisposing to this complication arise some concerns which can be elucidated by the following presentation of a case recently observed by us. A 60 year old man was diagnosed as having a classic B-CLL (stage II according to Rai classification) in November 1990 and followed for 11 years until the progression to stage IV. The molecular analysis of genes encoding for the variable region of immunoglobulin heavy chains (IgVH) revealed a mutated status (VH: 4-61; D: 7-27; JH: 4; % of mutation: 5.4; CD38 and ZAP-70 determined by flow cytometry were negative and FISH analysis showed no abnormalities. He underwent standard treatment with fludarabine achieving a complete response (CR) consolidated (January 2002) by four weekly standard doses of rituximab. CR was maintained until May 2006 when the patient presented general malaise, emaciation, mild splenomegaly without palpable lymph nodes and severe hypoproteinemia. A CT-scan revealed multiple deep enlarged nodes. A trephine bone marrow biopsy showed histological and immunohistochemical findings of a classic HL arising in CLL, so that a rare variant of Richter syndrome with HL features was diagnosed (Figure 1).

![Figure 1](image-url)
is an uncommon event, occurring in 0.4% of CLL patients and portraying a very poor clinical course with a reported median overall survival (OS) of 0.8 and 4.0 years, respectively. Most HL transformations of CLL developed in patients who have received previous treatments, mainly fludarabine or other purine analogs and rituximab (Timberdou AM et al, Cancer 2006). So, treatments given for CLL represent a crucial risk factor for the development of the secondary HL. This severe complication, paradoxically, occurs only in CLL patients with clinically most favourable biological features, such as the IgVH mutated status and the lack of ZAP-70 expression, which have been strongly associated with longer PFS and OS compared to unmutated and ZAP-70 positive CLL patients (Del Principe et al., Blood 2006), among which HL transformation has not been reported until now. Indeed, HL, which doesn’t express ZAP-70, originates from IgVH mutated and ZAP-70 negative B-cells, thereby, the progression to HL may occur only in patients with a mutated ZAP-70 negative CLL, which is provided by itself of a favourable course and a long survival, as observed in our case. Therefore, given the poor clinical outcome of the HL transformation occurring in an otherwise good prognosis CLL, and the key role of the treatments in its development, the decision to treat a ZAP-70 negative patient should be carefully evaluated, avoiding any early interventions if they aren’t absolutely mandatory.

**PO-137 IMMUNOGLOBULIN VARIABLE HEAVY-CHAIN (IG VH) MUTATIONAL STATUS AND TUMOR MICROENVIRONMENT INTERACTIONS IN B-CELL CHRONIC LYMPHOCYCTIC LEUKAEMIA (CLL)**

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**Introduction.** A prominent role in microenvironment-mediated survival of chronic B-CLL cells has to be ascribed to cell-cell contacts and to the engagement of surface receptors, such as CD40. The engagement of this molecule by its ligand, CD154, transduces a survival signal which is mediated by the nuclear translocation of NF-kB. B-CLL consists of 2 clinical entities with either somatically hypermutated (M) or unmutated (UM) IgVH regions. We found that M and UM B-CLL cells have different interactions with tumor microenvironment. UM B-CLL, but not M B-CLL, have a high rate of spontaneous apoptosis in vitro, are largely dependent from extrinsic stimuli of survival, such as IL4 and bone marrow stromal cells (BMSCs) and are susceptible to the action of agents which specifically interfere with CD40-CD154 interactions. **Methods.** Peripheral blood mononuclear cells from M and UM B-CLL patients were cultured either alone or with IL4 2 ng/ml or in co-culture with BMSCs and then evaluated for cell viability and apoptosis rate by Annexin V/Propidium Iodide staining and cytofluorimetric analysis. The role of CD40-mediated survival signalling in M and UM B-CLL was explored by culturing B-CLL cells from M and UM patients, in the absence or the presence of extrinsic survival stimuli (IL4 or BMSCs), with a sytetic antagonistic peptide which specifically blocks the CD40-CD154 interaction. **Results.** M B-CLL have a low rate of spontaneous apoptosis in vitro and survival of clonal B cells is largely independent from the presence of soluble factors (i.e. IL4) and cellular components, such as BMSC lines and BMSCs derived from B-CLL patients. On the contrary, UM B-CLL cells spontaneously undergo to apoptosis in the absence of extrinsic survival stimuli. In vitro exposure of UM B-CLL to cells a CD154-derived antagonistic peptide determines a significative reduction in the absolute number of viable clonal B cells and a consistent increase in the percentage of apoptotic cells, in the presence of IL4 and BMSCs. **Conclusions.** Defective apoptosis of UM B-CLL cells have to be ascribed not only to intrinsic defects of the neoplastic cells, but also to specific interactions of the malignant cells with the tumor microenvironment. Syntetic peptides targeting CD40-CD154 interaction have shown to abrogate survival signals provided by the tumor milieu in UM B-CLL and can be regarded as a promising strategy to overcome the mechanisms of drug resistance and to improve the outcome of high-risk patients.
PO-139
UNMUTATED IGHV AND HIGH RISK CYTOGENETIC ABNORMALITIES PREDICTING OUTCOME IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA TREATED AS FRONT-LINE CHEMOTHERAPY WITH ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE.

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The synergistic action of fludarabine with cyclophosphamide has demonstrated advantages as front line therapy in untreated patients with advanced chronic lymphocytic leukemia (CLL). The oral formulation of fludarabine showed a similar safety profile and response rate as the intravenous compound. Primary end-point of our study was to test efficacy and safety of the oral formulation of fludarabine combined with cyclophosphamide (FC) as front-line therapy in patients with progressive CLL. As secondary end-point we examined the impact of biological prognostic factors on treatment outcome. Thirty-five untreated patients with progressive CLL, with a median age of 69 years (52-75) received oral fludarabine (30 mg/sm) and oral cyclophosphamide (250 mg/sm) for 3 consecutive days every 4 weeks, for a total of 6 cycles. Thirty patients were in stage B/I with progressive disease, 1 in stage C/III, and 4 in stage C/IV. Fifteen patients had unmaturated and 14 mutated IgVH genes; in 6 patients the mutation status was not evaluable. Seven patients had the high risk cytogenetic abnormalities del(11q22.3) or del(17p-13.1). Fifteen patients had more than 20% ZAP-70 expression and 8 patients had more than 30% CD38 expression. Fourteen patients obtained complete response (40%) and 15 partial response (57%). The median time to progression after treatment (TTP) was 23 months, while the median time before starting a new therapy (TTR) was 38 months. Four patients died after progression of disease. Mild extra-hematological toxicity consisting of nausea and vomiting occurred in 13 patients during the treatment. Significant statistical differences were noticed in terms of overall response rate (ORR) (p=0.011), TTP (p=0.015) and TTR (p=0.044) between the group with high risk-cytogenetic abnormalities and the low-risk group. Significant statistical differences were noticed in terms of TTP and TTR between the IgVH mutated and unmaturated groups (p=0.034 and p=0.025). The over-expression of ZAP-70 or CD38 did not significantly influence the ORR, TTP, and TTR. Oral FC as front-line therapy in CLL achieved a good overall response rate in our series of patients. The haematological and extra-hematological side effect were mild. These results support the use of oral FC as front-line therapy in patients with favourable biological parameters. Patients with unmaturated IgVH genes and/or high risk-cytogenetic abnormalities should be considered for alternative more aggressive therapies.

Table 1. Response (%) in relation to patients characteristics.

<table>
<thead>
<tr>
<th>Group</th>
<th>All patients (n=40)</th>
<th>Failed fludarabine (n=25)</th>
<th>Failed rituximab (n=13)</th>
<th>Stage C (n=15)</th>
<th>ZAP-70 positive (n=23)</th>
<th>Unmutated cytogenetic (n=22)</th>
<th>1p- (n=9)</th>
<th>Age&lt;65 years (n=30)</th>
<th>Age≥70 years (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR</td>
<td>30.5</td>
<td>27.5</td>
<td>27.5</td>
<td>32.5</td>
<td>32.0</td>
<td>32.0</td>
<td>30.0</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>PR</td>
<td>56.0</td>
<td>28.0</td>
<td>32.0</td>
<td>32.0</td>
<td>26.0</td>
<td>40.9</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>SD</td>
<td>13.5</td>
<td>15.0</td>
<td>15.0</td>
<td>13.5</td>
<td>4.0</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PD</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
<td>46.7</td>
</tr>
</tbody>
</table>

PO-140
CLINICAL SIGNIFICANCE OF CD69 EXPRESSION IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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CD69 is expressed in all leukocytes during activation with rapid kinetic onset (2-4 hours) in lymphoid cells. Leukemic cells from all B-CLL cases exhibit features of activated and of antigen-experienced B lymphocytes based on the overexpression of the activation markers CD23, CD25, CD69 and CD71 (Damle, 2002). Moreover, the unmaturated B-CLL group shows a rapid disease progression and an inferior overall survival (Damle, Hamblin, 1999). Both CD69 and the unmaturated group resemble B cells at an earlier state of activation than the mutated group. The primary aims of our study were: 1) to determine progression-free survival (PFS) upon CD69 expression; 2) whether CD69 could predict varied outcome within mutated and unmaturated subgroups; and finally 3) whether CD69 was an independent prognostic factor. Therefore we investigated 237 pts, median age 65 years (range 37-87), 126 males and 111 females. With regard to modified Rai stages, 67 patients had a low stage, 161 an intermediate stage and 9 a high stage. CD69 was determined by a multicolor flow cytometric method fixing a cut-off value of 30%. CD69+ B-CLL patients were 72/237 (30%). CD69 ≤ 30% and Ig V gene mutated status were significantly correlated (69/87; p=0.00006). Furthermore, we found significant associations between lower CD69 and lower CD38 (133/179; p=0.005) or lower ZAP-70 (109/143; p=0.005). Besides, lower levels of soluble CD23 (sCD23) were associated with CD69 lower than 30% (118/148; p=0.00001). With regard to clinical outcome, a significant shorter PFS was observed in CD69+ pts vs CD69 negative pts (5% vs 56% at 14 years, p<0.00001, Figure 1) as well as in unmaturated pts vs mutated pts (7% vs 46% at 12 years, p<0.00001).

Figure 1.

To further explore the prognostic impact of CD69, we investigated its expression within unmaturated (39 pts) and mutated (87 pts) subsets. As a matter of fact, this activation marker identified two subsets of pts at different PFS within the mutated subgroup (57% for CD69 negative pts vs 9% for CD69+ pts at 9 years, p=0.00003). In multivariate analysis of PFS, ZAP-70 (p=0.0005), sCD23 (p=0.005) and CD69 (p=0.007), resulted to be independent prognostic factors. The early activation marker CD69 was very useful to identify pts at different progression rate, particularly within the mutated subgroup. Since the mutated subset consists of a heterogeneous population with a variable progression, CD69 antigen may be added to identify progressive pts and to take timely therapeutcic decisions.

PO-141
CELLULAR AND CIRCULATING LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND THEIR CORRELATION WITH BIOLOGICAL PARAMETERS IDENTIFYING HIGH-RISK PATIENTS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA.

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Information dealing with clinical relevance of serum and cellular expression of VEGF in CLL are lacking. With this in mind we analyzed
the correlation of both circulating and cellular VEGF levels with mutational status of IgVH, CD38 or ZAP-70 expression in two independent B-CLL patients’ cohorts. In 47 Binet stage A B-cell CLL patients, serum levels of VEGF measured at time of first diagnosis with an ELISA assay (VEGF Quantikine, R & D System) were significantly higher (median, 219 pg/mL; range, 36-2000 pg/mL) than controls (median, 142; range, 40-487 pg/mL; P<0.02; Mann-Whitney test) and could be used as an indicator of the density of BM (bone marrow) angiogenesis as suggested the close correlation with BM microvessel number (r=0.365; p<0.005). Furthermore, we found a positive association between VEGF circulating levels and mutational status of IgVH (p=0.005), CD38 (p=0.03) or ZAP-70 expression (p=0.03). In an independent patients’ set belonging to a cooperative database which accounts for 60 B-CLL patients, we investigated VEGF at gene expression level. To this purpose gene expression of purified cells from 60 patients were profiled using high-density oligonucleotide microarrays. Analysis of normalized expression values of VEGF gene transcript denoted a discrete expression in B-CLL cells. Interestingly, the pattern of distribution of gene expression level did not reflect either clinical stage (p=0.131) or biological variables of prognostic relevance such as mutational status of IgVH (p=0.678), CD38 (p=0.406) or ZAP-70 expression (p=0.06). In conclusion, our observations suggest that B-CLL cells express VEGF at cellular level, however, only the circulating counterpart which accounts for VEGF derived from both leukemic and accessory cells correlates with newer biological variables of prognostic relevance in CLL.

**PO-142**

**PROGNOSTIC VALUE OF SERUM MARKERS OF ESTABLISHED IMPACT ON PROGNOSIS IN RELATION WITH ZAP-70 EXPRESSION IN B-CLL PATIENTS**

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Introduction. A number of serum parameters such as thymidine kinase (TK), beta2microglobulin (B2-MG) and soluble CD23 (sCD23) have been considered prognostic markers in B-cell chronic lymphocytic leukaemia (B-CLL). Aim of this study was to assess the prognostic value of these parameters in our retrospective series of CLL patients in relation with ZAP-70 expression, tested by immunohistochemistry (ICH) in bone marrow (BM) biopsies. Methods. We measured the levels of TK, B2-MG and sCD23 in the sera, obtained at diagnosis and stored at −20°C, and evaluated ZAP-70 expression of leukemic cells in BM biopsies of 172 B-CLL patients, selected by the availability of both these samples at diagnosis. They were 107 males (62%) and 65 females, aged 38 to 85 years (median 65). At diagnosis 139 (81%) were Binet stage A, 26 (15%) B and 7 (4%) stage C. The median follow-up period from diagnosis was 63 months (range 6-246 months). Serum levels of TK activity were measured by a commercial radioimmunoassay and sCD23 levels by a commercial ELISA kit. Threshold for TK was considered 9.14 U/L (mean value of 80 healthy adults + 2SD) and for sCD23 was set at value of 60 U/mL. Of 30 normal samples was 1.24±0.34 U/mL). sCD23 in the sera, obtained at diagnosis and stored at −20°C, and evaluated ZAP-70 expression of leukemic cells in BM biopsies of 172 B-CLL patients, selected by the availability of both these samples at diagnosis.

**Table 1. Biological features of 100 concordant and 26 discordant cases for ZAP-70/IgVH mutation status.**

<table>
<thead>
<tr>
<th>Feature</th>
<th>ZAP-70+/IgVH unmutated</th>
<th>ZAP-70-/IgVH mutated</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Del(17p)</td>
<td>13/110</td>
<td>8/25</td>
<td>4/4</td>
</tr>
<tr>
<td>Del(11q)</td>
<td>10/87</td>
<td>8/25</td>
<td>4/4</td>
</tr>
<tr>
<td>Norm</td>
<td>30/247</td>
<td>25/77</td>
<td>16/21</td>
</tr>
<tr>
<td>Del(13q)</td>
<td>40/69</td>
<td>5/18</td>
<td>12/42</td>
</tr>
<tr>
<td>Del(12p)</td>
<td>26/21</td>
<td>16/57</td>
<td>8/31</td>
</tr>
<tr>
<td>Del(6q)</td>
<td>37/25</td>
<td>14/50</td>
<td>9/35</td>
</tr>
</tbody>
</table>

* FISH results not available in 2 cases

Raised B2-MG value was defined as ≥2.1 mg/L. ZAP-70 expression by leukemic cells was detected in BM biopsies by IHC using a mouse anti-human monoclonal antibody to ZAP-70 (clone 2F3.2, Upstate, Lake Placid, NewYork). Results. Results are reported in the table: all 4 prognostic parameters (serum levels of TK ≥9.14 U/L, sCD23 ≥60 U/mL, B2-MG ≥2.1 mg/L and ZAP-70 expression) were associated with shorter TTP, but only raised B2-MG and ZAP-70 positivity affected OS. At multivariate analysis only ZAP-70 positivity was associated both with shorter TTP and OS, elevated sCD23 levels with shorter TTP. The association with poorer TTP and OS was confirmed only for ZAP-70 positivity when we limited the analysis to the 139 Binet stage A patients. Median OS and TTP of 54 stage A ZAP-70 positive patients were 143 and 45 months, respectively, vs not reached of 85 stage A ZAP-70 negative. Mutational IgVH gene status was analysed in 62/172 patients (54 mutated and 28 unmutated). Concordance with ZAP-70 expression was found in 84% of cases. Conclusions. Although different serum markers appeared to be of prognostic relevance at univariate analysis, only ZAP-70 expression independently influenced survival in our CLL series, and particularly in Binet stage A patients.
IgVH status are mostly ZAP-70+/IgVH mutated, have a low incidence of poor risk genetic abnormalities del(17p)/del(11q) and a relatively long TH, suggesting that IgVH mutational status is more relevant than ZAP-70 in the clinical outcome of this subgroup. 

**PO-144**

**A BIASED DELETION IN A SUBSET OF VH3-21 CLL PATIENTS WITH HOMOLOGOUS HCDR3 SEQUENCES SUGGESTS A COMMON ANTIGENIC DRIVE MECHANISM**

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A subset of CLL patients using VH3-21 gene in association with V\textsubscript{lambda}-2-14 gene and carrying homologous heavy and light chain CDR3 sequences has been recently described in Northern Europe. In the Mediterranean area CLL using VH3-21 gene is much more rare and has been grouped into 2 subsets with different molecular and clinical features, according to the degree of homology in HCDR3 sequences. Interestingly, **CLL-biased** one codon deletion in the immunoglobulin heavy-chain CDR2 domain of V\textsubscript{H}-3-21 CLL patients has been recently described in Mediterranean cases, with a hot spot in codon 61 (serine). Moreover, a close association between this deletion and the subset of VH3-21 CLL with stereotyped receptors have been observed. The aim of our study was to analyze the molecular features of V\textsubscript{H}3-21-expressing cases derived from 198 patients with CLL referred to our Institution between November 2003 and April 2007. In the 9 V\textsubscript{H}3-21 cases we identified (4.7%), all of male gender, we investigated also the IgVH mutational status, the CDR3's configuration and the presence of subset-biased mutations. The heavy and light chain rearrangements analysis was performed by using reverse transcribed RNA from peripheral blood samples. A clonal IgH gene rearrangement was demonstrated in 191/198 patients. Sequence analysis of rearranged genes demonstrated a somatic mutated pattern in seven of nine cases (78%). Six patients (66%) had short homologous HCDR3, whereas the remaining three shared totally unrelated HCDR3 all showed rearrangement of the JH\textsubscript{6} gene and displayed clonal lambda chain expression. Moreover, a restricted V\textsubscript{lambda}-2-14 gene usage with homologous light-chain CDR3 sequences was demonstrated in 75% of the tested cases (see Table 1).

Table 1. Molecular characteristics of the VH3-21 CLL patients with homologous HCDR3.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mutation status</th>
<th>Heavy-chain CDR3*</th>
<th>JL gene</th>
<th>V-lambda</th>
<th>Light-chain CDR3**</th>
<th>Heavy-chain CDR2 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL1</td>
<td>unmutated</td>
<td>ARGANGMDV</td>
<td>J6</td>
<td>V\textsubscript{lambda}-34</td>
<td>QWQSSSHPH</td>
<td>codon 63</td>
</tr>
<tr>
<td>CLL2</td>
<td>mutated</td>
<td>ARGANGMDV</td>
<td>J6</td>
<td>V\textsubscript{lambda}-34</td>
<td>QWQSSSHPH</td>
<td>none</td>
</tr>
<tr>
<td>CLL3</td>
<td>mutated</td>
<td>ARGANGMDV</td>
<td>J6</td>
<td>V\textsubscript{lambda}-34</td>
<td>QWQSSSHPH</td>
<td>codon 63</td>
</tr>
<tr>
<td>CLL4</td>
<td>mutated</td>
<td>ARGANGMDV</td>
<td>J6</td>
<td>V\textsubscript{lambda}-19</td>
<td>QWQSSSLSH</td>
<td>codon 63</td>
</tr>
<tr>
<td>CLL5</td>
<td>mutated</td>
<td>ARGANGMDV</td>
<td>J6</td>
<td>$</td>
<td>$</td>
<td>codon 63</td>
</tr>
<tr>
<td>CLL6</td>
<td>mutated</td>
<td>ARGANGMDV</td>
<td>J6</td>
<td>$</td>
<td>$</td>
<td>codon 63</td>
</tr>
</tbody>
</table>

| light chain sequencing still in progress | amino acids changes in the conserved sequence ARGANGMDV are reported in bold type | amino acids changes in the conserved sequence QWQSSSHPH are reported in bold type |

Interestingly, an identical deletion occurring at codon 63 (isoleucine) of the HCDR2 domain was observed in 4 of 6 patients (67%). The prevalence of homologous VH3-21 CLL patients in our cohort (3.1%) is similar to that described in Northern Italy by a recent multicenter study. The recurrent deletion of codon 63 in the HCDR2 domain of our VH3-21 CLL patients, only in the subset displaying homologous HCDR3, strongly suggests the presence of an antigenic drive in the pathogenesis of VH3-21 CLL patients.

**PO-145**

**LYMPHOPROLIFERATIVE DISEASE AND ACQUIRED C1 INHIBITOR DEFICIENCY**

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Acquired C1-inhibitor (C1-INH) deficiency with angioedema (AAE) is a rare syndrome frequently associated with lymphoproliferative diseases and/or anti-C1-INH inactivating auto-antibodies. Lymphoproliferative disorders in AAE patients, span from monoclonal gammopathies of uncertain significance to non Hodgkin lymphoma (NHL). In addition, auto-antibody to C1-INH, can be considered as a sign of a chemotherapy, and the cell is in remission after 4 years of follow up. In the remaining 5 patients the disease is stable without therapy. This report confirms that the risk of NHL is markedly increased in patients with AAE. The variety of clinical presentation and response to therapy of NHL suggest that the course of B cell malignancies in these patients has no specific characteristics. The same seems to be true for MGUS, which does not progress to multiple myeloma with increased frequency.

**PO-146**

**MUTATIONAL STATUS, ZAP-70 AND CD-38 AND THE RISK OF PROGRESSION IN 142 PATIENTS WITH EARLY STAGE CLL**


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**Introduction.** Somatic mutations in the immunoglobulin heavy-chain gene of the CLL cell (mutational status) is considered as one of the most predictive factors for the risk of progression in patients with early stage CLL. CD-38 and ZAP-70 were also shown to have a similar prognostic value and they were sometimes considerate as a surrogate markers of mutational status. This study was aimed to evaluate the prognostic impact of different prognostic factors on time from diagnosis to initial treatment in this subgroup of CLL patients. METHODS We studied 142 patients (82 M; 60 F); median age was 66 (range 56-85). They all had a stage A CLL at diagnosis. When Rai stage was considered, 90 had stage 0, 57 stage I and 15 patients stage II. Median follow-up was 48 months (range 3-245 months). Absolute lymphocyte count was: median 12.14×10\(^9\)/L (range 2.5-199). Median hemoglobin was 13.82 g/dL (range 11.3-17.5 g/dL). Median platelet count was 198×10\(^9\)/L (range 101×10\(^9\)/L to 553×10\(^9\)/L). Patients were treated when symptomatic or progressive disease developed. The following cut-off values were used: CD-38 >20%; ZAP-70 >20%; VH unmutated >98% homology with germ-line sequence. 32% of patients were CD38 positive, 29% were
ZAP-20 positive, 25% patients were VH unmutated. Results. 49/142 patients included in the study had received one or more chemotherapy regimens. Median interval between diagnosis and initial therapy was 29 months (range: 0-193). 74% of ZAP-70 positive were treated vs 23% of ZAP-70 negative. 61% of unmutated were treated vs 26% of mutated pts. 59% of CD-38 positive were treated vs 35% of CD-38 negative. When the interval from diagnosis to therapy was analyzed with a Kaplan-Meier survival curve (see figures), mutational status and ZAP-70 positivity were the most significant prognostic factors. Median treatment-free time was 40 months for mutuated vs. 190 months for mutuated patients ($p=0.002$). When CD-38 was analyzed, a borderline significant value was obtained ($p=0.08$). Absolute lymphocyte count (with a cut-off at 15 cells/10^9/L) also showed a significant impact on the need of treatment. Discussion. Our results confirm mutational status as the most valuable prognostic factor for identifying, among early stage CLL, patients who will need therapy.

**PO-147**

**MOLECULAR ANALYSIS OF REARRANGED IMMUNOGLOBULIN HEAVY CHAIN GENES IN LYMPHOPROLIFERATIVE DISEASE**


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Introduction. Most lymphoid malignancies with B-cell origin display preferential immunoglobulin heavy chain gene usage. Biased immunoglobulin heavy chain (IGH) gene usage has been reported in different entities of B-cell lymphoma, B-lineage acute lymphoblastic leukaemia (ALL) and in B-cell chronic lymphocytic leukaemia (CLL). This non random usage of individual VH, DH, and JH genes has been suggested to reflect that the immunoglobulin structure may play a role in leukaemia/lymphoma development, possibly through unknown antigen stimulation. Few studies have done comparing different adult human leukaemia of the same geographic area: so we examined the immunoglobulin (IG) VDJ heavy chain usage and somatic mutation pattern in malignant B-cells of 101 unrelated patients. Methods. VDJ clonal rearrangements were obtained in 36 B-ALL, 48 CLL and 17 Non Hodgkin Lymphoma (NHL) from our cohort of patients. DNA amplification of rearranged IG heavy chain genes was performed as described in BIOMED 2. PCR products were directly sequenced (BigDye Kit Applied Biosystems) and analyzed by an automated sequencer (ABIPRISM 310 Applied Biosystems). Sequences were compared to those in the online IMGT or NCBI IgBlast databases. Results. We examined the IG genes used in 59 clones of 48 patients with CLL (mutational status: 53.9% mutated, 66.1% unmutated). The most commonly expressed VH gene was VH1 (34.4%), followed by VH3 (33.9%) and VH4 (25.4%); collectively these genes accounted for 93.7%. We find a preferential use of the VH-69 exclusively in unmutated CLL. In our series the joining family JH6 occurs as most common (57.2%) and is followed by JH4 (40.3%). The most common DH gene was DH3 (55.6%). A total of 48 IGH sequences were identified from 36 cases of B-ALL VH3 (41.7%) VH4 (20.8%) and VH1 (16.7%) amounted to 79.2% of rearranged families. Usage of D2 and D3 families was most prominent (41.7%) VH4 (20.8%) and VH1 (16.7%) amounted to 79.2% of rearranged families. Usage of D2 and D3 families was most prominent (39.6% and 27.1% respectively). JH6 occurred most commonly and was followed by JH4 (30.5%). The most common DH gene was D3 (35.6%). A preferential usage of VH3 rearrangements in adult ALL and of VH4 in NHL. D3 family genes was prominent in LLc and NHL D2 in B-ALL ($p=0.068$). There was a predominance of the JH4 gene in NHL of JH6 in B-ALL. This results confirm the observation that, VH DH JH genes expressed in lymphoproliferative disease, are highly selected, peculiar in different disease and not representative of IG expressed by naive B cells, also in our geographic area.

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**PO-148**

**LYMPH NODE ENLARGEMENT IN ADULT PATIENTS REFERRED FOR A SUSPECT OF HEMATOLOGICAL MALIGNANCY**


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Introduction. The diagnostic approach to lymphadenopathy (LYA) frequently challenges the knowledge and the experience of the primary care doctor and of the specialist – usually a hematologist. Published diagnostic guidelines are still lacking. Color doppler ultrasound has been largely applied to provide useful information in the differential diagnosis of malignant versus lymphoid LYA. The aim of this study was to retrospectively evaluate the incidence and causes of LYA in adult patients referred to the hematologist for a suspect of hematological malignancy.

Methods. We retrospectively evaluated 550/7200 (7.6%) consecutive patients referred at our Hematology division because of LYA. According to our previous published criteria, patients showing superficial LYA underwent laboratory examinations and instrumental investigations (Cancer 1999; 85, 2480). Patients showing abnormal laboratory values and a diagnostic work-up of the enlarged lymph node referring to non malignant LYA underwent a bone marrow needle aspiration/biopsy and/or lymph node biopsy. Results. Over a two year period, we evaluated 29% of our patient population by lymph node biopsy. Hystopathological lymph node examination allowed to identify the causes of the lymph node enlargement as follows: 51.9% non-Hodgkin and Hodgkin lymphomas (LNH and HD), 18.5% metastatic solid tumor lymph node involvement, 21.5% inflammatory/reactive lymph nodes, and 7.5% infective and granulomatosis diseases; in the remaining 1.2%, the biopsy revealed the presence of a neoplasia. Among the 390 patients in which the lymph node biopsy was not performed, 94.2% of them showed a spontaneous resolution of the lymph node enlargement during the clinical follow-up (1-3 months), 2.5% were classified according to the bone marrow biopsy as low grade LNH, 2.8% were characterized by a miscellaneous of solid tumors, 1.2% of patients which underwent following further diagnostic non-invasive examinations, and 0.5% were identified as metastatic solid tumors by both clinical and instrumental examination. Discussion. In conclusion, we performed an histopathological diagnosis in one third of the cases while in the remaining cases a clinical work-up and follow-up confirmed the presence of a non-malignant LYA. This retrospective study contributes to define criteria capable of distinguishing in patients with LYA those which require a lymph node biopsy aimed at diagnosing a malignant LYA.

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**PO-149**

**CORRELATION BETWEEN CYTOPHLOMORIMETRIC CHARACTERISTICS AND CYTOGENETIC ABNORMALITIES IN CLL PATIENTS**


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Cytogenetic abnormalities, ZAP70 and CD38 expression represent important prognostic factors for the management of chronic lymphocytic leukaemia (CLL). From January 2005 to September 2006, 78 consecutive patients affected from CLL were referred to our Department for study by FISH of cytogenetic abnormalities (trisomy 12, del 11q22-23, del 17p13 and del 13q14) and of CD 38 and ZAP 70 expression by cytophlorimetric analysis. The median age of the patients was 64 years (40-79 years); 50 male and 28 female. 10 pts were at diagnosis, 68 with previous CLL diagnosis, 35 on treatment. Clinical staging of B-CLL patients showed that 68 pts were in stage A Binet, 9 in stage B and 1 in stage C. Median WBC count, was 18,450×10^9/L. (range: 4,700-449,000). CD 38 was positive in 19/78 pts (24%) and ZAP 70 was positive in 37 pts (47%). The cumulative incidence of cytogenetic abnormalities was 67%. The most common abnormality was del 13q (54%) observed as isolate in 34 pts and in association with other abnormalities in 6 pts. Del 11q was observed in 7 pts (9%), trisomy 12 in 6 pts (8%) and del 17p in 5 pts (6%). The cytogenetic aberrations with unfavourable prognostic significance, especially del 11q, were associated with ZAP 70 (52% vs 14%; $p<0.07$) and CD38 positivity ($p=0.04$) ($p=0.005$). On the other hand a significant association was observed between CD38 negativity and del 13q ($p=0.02$). Moreover CD38 and ZAP 70 expression were signifi-
cantly correlated together (p = 0.002). In the 33 pts already treated, a higher incidence of del 11q, del 17p and trisomy 12 was observed (p = 0.007), while in untreated patients a higher incidence of isolate del 15q was found (p = 0.02). No correlation between ZAP 70 and treatment was observed. In our experience the most significant correlation appear to be between cytogenic abnormalities and CD38 instead of ZAP-70 as reported by the majority of the other studies.

**PO-150**

**IMPACT OF B2 MICROGLOBULIN (B2M) AND LDH FOR PROGNOSTICALLY CLUSTERING CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL) CASES IN THE ERA OF BIOLOGIC RISK FACTORS**


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Background. B2M and LDH are well described prognostic factors in CLL. More recently, the high predictive power of IgVH gene status, CD38 and ZAP-70 expression have been reported. Nevertheless the relative prognostic impact of the individual parameters needs to be further investigated. Aim. The purpose of this study was to assess the individual prognostic power of B2M and LDH level, IgVH mutational status, CD38 and ZAP-70 expression with respect to time to treatment (TTT) in a cohort of 251 B-CLL. Patients and methods. The median age of the patients was 64 years; 36% were females; 86% were classified as Binet stage A, 11% stage B and 3% stage C. At baseline, the serum B2M and LDH level have been dosed and CLL neoplastic cells were evaluated for ZAP-70 and CD38 expression and IgVH mutational status. In particular, ZAP-70 expression was investigated by Western blot and cases were classified as ZAP-70 strong, ZAP-70 weak and ZAP-70 neg. Results. Elevated B2M and LDH serum levels were detected in 42% and 12% of cases, respectively. A statistically significant shorter TTT was demonstrated in CLL cases with elevated serum levels of both B2M and LDH (5-years TTT 82% for normal B2M versus 60% for elevated B2M, p<0.0001; 5-years TTT 76% for normal LDH versus 48% for elevated LDH, p<0.0001). At univariate analysis, biologic risk factors such as CD38 (categorised according to cut-off point of 30%), IgVH gene status (considering the classical VH homology cut-off value of 98%), ZAP-70 (classified as negative, weak and strong) stratified CLL cases with statistically significant different TTT: 5-years TTT 87% for CD38<30% versus 45% for CD38≥30%, p<0.0001; 3-years TTT 94% for ZAP-neg cases, 82% for ZAP-70 weak and 58 for ZAP-70 strong, p<0.0001; 5-years TTT probability: IgVH mutated versus IgVH unmutated: 86% versus 59%, p<0.0001. Among other clinical and haematological parameters, only the Binet stage showed a prognostic impact. At Cox multiple regression model, B2M, Binet stage, IgVH status and CD38 still retained an independent prognostic factor (B2M: Exp(B) 2.57, p=0.002, Binet stage: Exp(B) 2.7, p<0.0001; IgVH: Exp(B) 0.83, p=0.04; CD38 Exp(B) 1.0, p=0.001). Conclusions. This study showed an independent predictive power of B2M, also when its prognostic value was considered along with strong biological parameters such as IgVH, CD38 and ZAP-70. However, we should take into account that B2M, a typical neoplastic mass marker, could modify during the course of the disease. Therefore, its prognostic value could be less significant in anticipating clinical outcome in CLL in comparison with the parameters strictly associated with cell biology, likely more stable over the time.

**PO-151**

**THE CLINICO-BIOLOGICAL FEATURES OF A-B-CLL VARIANT, DEFINED ACCORDING TO A COMBINED CYTOFLUORIMETRIC/FISH DIAGNOSTIC APPROACH**


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In the present study, we describe the clinico-biological features of 63 cases of variant B-CLL (v-B-CLL) defined according to previously described diagnostic algorithm for CD5+ mature B-cell leukemias, based on the combined use of Cytofluorimetric (CFM) analysis and t(11;14)(q13;q2) detection by means of fluorescence in situ hybridisation (FISH). These leukemias were characterized by S IgVH (+) /CD23- or CD23+, CD79b/CD20+ and negativity for t(11;14)(q13;q2). A historical series of 112 classical B-CLL was used as comparison. The mean age was 61 years (53-85) and M:F ratio 1.9. The v-B-CLL cases were significantly different from the cases in the classical B-CLL. The median overall survival was 80 months in v-B-CLL pts while it was not reached in B-CLL pts; 37 pts (57.7%) in the first group started chemotherapy vs 84 pts (56.4%) in the latter. The analysis of leukemia cell biological characteristics related to the prognosis was also made in part of the cases. A classic or mixed-CLL cytomorphology was seen only in 22/61 pts (36%). The percentage of CD38 positive cases was higher in v-B-CLL (60.5%) than in B-CLL (44.6%, p=0.025), the IgVH mutation status, evaluated in 30/63 cases, was more frequently hypermutated in v-B-CLL group (23/30) than B-CLL (p=0.03); the percentage of CFM ZAP-70 positivity, evaluated in 42/63 cases, was similar in two groups (47.6% vs 57.4%, respectively). Interestingly, significant differences were found about the frequency of the recurrent chromosome alterations, evaluated in 48/63 cases, by means of FISH analysis: trisomy 12 was more frequent in v-B-CLL (57.5% vs 18.4%, p=0.02), while del1q14 was more frequent in B-CLL (14.5% vs 37%, p=0.007). The presence of splenomegaly was 37% in v-B-CLL and 27% in B-CLL, p=0.007. In conclusion, our study identifies on the basis of a defined CFM-FISH diagnostic approach, a variant form of B-CLL that shows significant differences in terms of genetic and clinical features.

**PO-152**

**ANALYSIS OF CD200 EXPRESSION IN B CELLS LYMPHOPROLIFERATIVE DISORDERS**

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CD200 is a highly conserved type I transmembrane 40-45 kDa glycoprotein formerly known as OX-2. It has been demonstrated that interaction of CD200 with its receptor expressed on macrophages induces a downregulation of macrophages activity suggesting a regulatory function. Recently, expression of CD200 has been recorded on thymocytes, activated T cells, B cells, dendritic cells, endothelial cells and neurons, but not on NK cells, monocytes, granulocytes and platelets; in addition it has been demonstrated that CD200 expression on Myeloma plasma cells represents a negative prognostic factor for this disease. In the present study we analyze the expression of CD200 antigen in lymphoproliferative disorders by flow cytometry (FACScalibur, BD Biosience) using a direct immunofluorescence technique. In all cases CD200 expression was carried out using three color staining. With the aim to better identify this molecule in neoplastic and normal lymphocytes we performed a CD19/CDS/CD200 combination and the analysis was conducted on
lymphocytes population gated by FSC and SSC. CD200 expression was evaluated in 55 samples: 12 were B chronic lymphocytic leukaemia (B-CLL), 4 were Mantle Cell Lymphomas (MCL), 4 were Low Grade Lymphomas (LGNL), one case was Hairy Cell Leukemia (HCL) and 10 derived from normal subjects. As previously reported, the majority of normal B lymphocytes showed CD200 with a variable intensity (median MPF 51), while only a little percentage of T lymphocytes was CD200 positive. CD200 antigen was expressed in neoplastic cells of 12/12 B-CLL cases and its intensity was more elevated compared to normal B cells (median MPF174). On the contrary, CD200 was expressed in 1/6 (16.6%) neoplastic cells of MCL cases, and 3/4 (75%) of LGNLH. CD200 expression was recorded also on hairy cells with a reduced intensity with respect to both normal B lymphocytes and B-CLL. It is interesting to note that the MCL case CD200 positive expressed CD20 antigen at low intensity, as in B-CLL. Since previous data reported the evidence of a role for CD200 in the regulation of immune system, our preliminary results could be the start for further studies to explain the immune system deficiency documented in B-CLL.

PO-153
RITUXIMAB FOR THE TREATMENT OF B-PROLYMPHOCYTIC LEUKEMIA

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Prolymphocytic leukaemia (PLL) is a rare lymphoproliferative disorder characterized by marked leukocytosis and splenomegaly. PLL accounts for approximately 2% of chronic lymphoid leukemias. The clinical course is progressive in the majority of cases due to the resistance of the disease to conventional chemotherapy. In previous decades, splenectomy, splenic irradiation, leupheresis, and alkylating agents by alone or in combination with other cytotoxic agents have been used for the treatment of PLL. Subsequently, purine nucleoside analogs (fludarabine, cladribine, and pentostatin) have been introduced for the therapy of these disorders. More recently, monoclonal antibodies, especially alemtuzumab in T-PLL, have been found effective in a few cases. We report the clinical experience of 12 patients treated successfully of B-PLL cell with rituximab reported in the literature. A 73 years-old man was admitted to our Hospital in November 2004 because of lymphocytosis and splenomegaly. PLL diagnosis showed only splenomegaly without lymphadenopathies. Laboratory investigations showed: WBC 18.300/μl/mq (Ly 50 per cent), LDH 134 units/liter, beta 2 microglobulina 4,7 milligrams/liter. Monocolonal component IgG kappa in the serum was detected. The bone marrow aspiration showed an increase of lymphocytes of middle size with prolymphocytic habitus. These cells were: CD19+, CD 20+, CD5+, CD23-CD10-CD79b+, IgD+ IgM-. The cyogenetic examination showed a complex Karyotype with the presence of two pathological correlated clones: 45,XY,der(3)(pter->3q29::3q29->3q25), derivative clone and 45,XY,der(13)(13)/45, idem,der (13;13)(q10;q10). The median time of recovery for neutrophil count major1500/mmc was 17.5 days(range 15-31) for platelets count major35000/mmc was 20days (2-21), and hemoglobin level major 8 g/dl was 27 days. Our median follow up at today was 275days(range 40-510). All patients are in hematological and morphologic complete remission. Conclusion. Use of Rituximab in first line therapy, despite its use in resistant/relapsed disease, is safe and feasible. Moreover when Rituximab is used in first line therapy, median time of recovery in neutrophils and platelets count is significantly shorter than that obtained after therapy with 2CdA alone (8 and 4 weeks respectively).

PO-154
RITUXIMAB AND 2CdA IN HCL FIRST LINE TREATMENT: A FASTER WAY TO REMISSION. REPORT OF FOUR CASES

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Background. Hairy-cell leukemia (HCL) is an uncommon B-cell chronic lymphoproliferative disorder that accounts for about 2% of all leukemias. Although the disease is generally indolent in its natural course, the majority of patients require treatment for life-threatening infections due to pancytopenia or symptomatic splenomegaly. Remarkable progress has been made in the treatment of HCL. Prior to the advent of nucleoside analogues, which are currently the standard initial treatment, interferon and splenectomy were the most effective therapies. Treatment with purine analogs (PNA) such as 2-chlorodesoxyadenosine (2-CdA) is associated with excellent remission rates and long-term survival. Recently, immunotherapeutic approaches which use monoclonal antibodies, like rituximab, have increased the number of therapeutic options for HCL and offer promising salvage strategies for patients who relapse or who are refractory to treatment with purine analogues. In previous study rituximab used in patients relapsing or refractory to CdA and for elimination of MBD after treatment with PNA showed hematological CR and good MRD eradication. Methods. We analyzed, in a retrospective study, four consecutive male patients, 3 with HCL, and one with HCLV treated at diagnosis with once rituximab administration at dose of 375 mg/mq and 2CdA at dose of 0.14 mg/mq/day s.c. for 5 days. Their median age was 71years(range 44-71). All patients showed splenomegaly and thrombocytopenia (median platelets count was 10000/mmq and range 6000-8000/mmq), that of these presented neutropenia (median neutrophils count was 440/mmq with range 430-500/mmq) and one patient evidenced anemia (Hb inferior 8 g/dl). Results. None of patients had extra-hematological therapy-related side effects. Three patients showed viral infection after therapy, although received prophylaxis with aciclovir. The median time of recovery of neutrophil count major1500/mmq was 17.5 days(range 15-31) for platelets count major10000/mmq was 20days (2-21), and hemoglobin level major 8 g/dl was 27 days. Our median follow up at today was 275days(range 40-510). Actually all patients are in hematological and morphologic complete remission. Conclusions. Use of Rituximab in first line therapy, despite its use in resistant/relapsed disease, is safe and feasible. Moreover when Rituximab is used in first line therapy, median time of recovery in neutrophils and platelets count is significantly shorter than that obtained after therapy with 2CdA alone (8 and 4 weeks respectively).

PO-155
CHROMOSOME ABNORMALITIES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA DETECTED BY INTRA PHASE FLUORESCENCE IN SITU HYBRIDIZATION: CORRELATION WITH BIOLOGICAL FEATURES

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Background. B-CLL has a variable history with respect to time to progression and response to standard cytotoxic therapies. FISH interphase cytogenetic analysis is superior to standard karyotype analysis in identifying known abnormalities and is able to reveal chromosome changes in the majority of B-CLL samples (1,2). The most common abnormalities are del (13) (q14.3), del (11)(q22–23), trisomy 12 and del(1)/p13.1. Del(11q) and del(17p) are definitely associated with a bad prognosis(1). Some Authors have correlated FISH features with other biological parameters, including sex, age, Rai stage, IgM/huatalional status and CD38 expression (3). We tried to establish such correlations in a series of patients coming from several Centers in Romagna. Materi

References
**CD5-NEGATIVE CLONAL B LYMPHOCYTOSIS: ARE THEY CLL?**


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Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries, and is characterized by accumulation of neoplastic B-lymphocytes in bone marrow, lymphoid tissues and peripheral blood. Markers universally associated with B-CLL are CD5, CD23 and low expression of clonally restricted surface immunoglobulin. Even if some investigators have doubts about the existence of CD5 negative (CD5-) B-CLL, lymphoproliferative disorders clinically indistinguishable from classic CD5+ CLL but which not showing typical surface markers have been described. We reanalyzed by a modern technology (FACSCan to instrumentation and six-color flow cytometry) 13 patients previously classified as CD5- lymphoproliferative disease with predominant lymphocytosis and CLL-like features. Ten of 13 patients re-examined, although not showing marked lymphoadenomegaly, were re-classified by flow cytometry as marginal zone or mantle cell lymphomas. In 2 out of these 10 cases atypical morphology in peripheral smear was present. Among the three remaining patients, one case showed two lymphocyte subpopulations (CD5+ and CD5-) and was classified as composite lymphoma. Another patient had a level of CD5 only slightly higher as compared to the cut-off point of the technique, and was classified as CD5± CLL. Finally, a single case showed all clinical and cytometric features of classic CLL, except for CD5, and therefore was the unique to be diagnosed as having true CD5- CLL. In all patients the evaluation of cytoplasmic CD5 confirmed the data of surface antigen expression. CLL is a complex and heterogeneous disease; our study sprang from the need to identify and classify in unequivocal way CD5- lymphoproliferative disease with prevalent lymphocytosis. Our data showed large heterogeneity of clinical, morphological and cytofluorimetric findings. The possibility of comparing different inter-laboratory results will contribute to decrease difficulties in classification and incidence estimation of this uncommon disorder.

**References**


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**Table 1.**

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<th>N° Pts</th>
<th>N° Fts</th>
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<td>8</td>
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<td>15.8 (4.8-137.3)</td>
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<td>8</td>
<td>12</td>
<td>7/1</td>
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<td>16.7 (17.4-40.9)</td>
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<td>10 (7.9-49.2)</td>
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**Time to treatment (mos)**

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**References**

and the their PS ranged from 1 to 2. One patient, with matched donor available, received the therapy to reduce the cutaneous and circulating Sezary’s cells before the conditioning treatment. Results. All patients responded to the bortezomib-IFN association: the first treated patient is in CCR 13 weeks after the induction phase evaluation, the other patients with a short period of treatment (7-8 weeks) achieved a good partial remission (with disappearance of cutaneous symptoms; reduction >55% of circulating Sezary’s cells). The protocol was well tolerated and any toxicity has been experienced. Conclusions. The association bortezomib-IFN seems effective and safe in reducing tumor burden in advanced SS patients. A longer follow up and a higher number of treated patients are requested for the response evaluation.

**PO-158**

**LONG-TERM SUBCUTANEOUS ADMINISTRATION OF ALEMTUZUMAB (CD52) MONOCLONAL ANTIBODY IN A HEAVILY PRETREATED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENT**

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**Introduction.** Alemtuzumab (Mab Campath) is a humanized monoclonal antibody against CD52 antigen, expressed in most human B and T lymphocytes. The drug has shown considerable activity in both relapsed/refractory chronic lymphocytic leukemia (CLL) and in the front line setting, to treat residual disease following purine analogue-based treatment. Adverse events usually include acute first dose reaction, hematological toxicity and infectious complications, particularly cytomegalovirus (CMV) reactivation. Therefore, antiviral and antibacterial prophylaxis is mandatory. Initial studies employed alemtuzumab by intravenous route; however, more recently, subcutaneous (s.c.) route has been demonstrated to be better tolerated and as effective as i.v. administration. Here we report our experience of long-term s.c. alemtuzumab in a CLL patient with progressive disease after several chemotherapy regimens. Case Report. B lineage CLL Stage III Rye was diagnosed in April 2002 in a 57 year old man (wbc 88.000 /spleen/nodes>3 cm all stations). He subsequently received four lines of therapy, with relapse after every course. Two courses of four anti-CD20 (Rituximab) infusions were also given after chemo, as well as high dose immunoglobulins and corticosteroids for two episodes of immune haemolytic anaemia. Alemtuzumab was started in December 2005 for progressive disease, and given at 30 mg s.c. 3 times a week, after the usual first week of stepped up dosing, for 6 weeks. A partial hematological response was achieved and maintenance therapy with 15 mg s.c. every 2 weeks was then given, which is still ongoing for more than 15 months. Weekly surveillance for CMV by qualitative PCR-based assay was done and infection prophylaxis (trimethoprin-sulfamethoxazolo/acyclovir/itraconazol) as well as i.v. immunoglobulins (10 gr q 21 days) were given for 6 months. Initial grade I-II local skin erythema and injection site pain disappeared in four weeks. Symptomatic CMV reactivation, with fever and increase of aminotransferase values, occurred after one month of therapy, which resolved after a 4 week interruption of alemtuzumab treatment and valacyclovir (450 mg qd) therapy. No more infections or episodes of haemolytic immune anaemia occurred and the patient maintained a good partial hematological remission, with normal blood counts and persistent 8 cm abdominal lymphadenopathies. The patient is now still well (karnowsky 100%) in persistent partial remission. Discussion. In conclusion, alemtuzumab has been safety administered for more than 16 months to a poor prognosis CLL patient, with prolonged disease control. Long-term alemtuzumab treatment by low dose, subcutaneous administrations may have a favourable toxicity profile and may provide prolonged disease control and survival to poor prognosis, heavily pretreated CLL patients.

**PO-159**

**CHEMOTHERAPY AND ANTIVIRAL THERAPY IN HHV8-, HIV NEGATIVE ASSOCIATED MULTICENTRIC CASTLEMAN DISEASE: A CASE REPORT**

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Multicentric Castleman disease (MCD) is an aggressive variant of a rare lymphoproliferative disorder associated with Human herpes virus 8 (HHV8) infection in up to 50% of cases and characterized by lymphadenopathy with angiofollicular hyperplasia and plasma cell infiltrations associated with severe systemic symptoms and HIV infection, characterized by a refractory disease. Recently there are same reports who showed that alemtuzumab is an effective agent in refractory AIHA. We report a case with a MCD associated with cutaneous Kaposi sarcoma in HIV negative patient. A 54-year-old woman with cutaneous Kaposi sarcoma showed diffuse lymphadenopathy. A diagnosis of Multicentric Hyaline-vascular variant Castleman disease was performed by lymphonodal biopsy. Bone marrow sample demonstrated only erythroid dysplasia. Tests for HIV and HHV8 infection were performed resulting negative for HIV and positive for HHV8 DNA. The patient underwent 4 courses with CVP chemotherapy and IFN immunotherapy 1.500.000 MUI 3qw. After the fourth course, the patient showed a progression of the disease characterized by multiple lymphadenopathy, severe pancytopenia, disseminated oedema, pulmonary and pericardial effusion and ascites. A bone marrow biopsy resulted massively compromised by Castleman disease. Considering the aggressiveness of the disease the patient underwent two courses of chemotherapy with modified IEV and peripheral blood stem cell collection was tried but unfortunately failed. A restaging was performed: CT demonstrated a good partial response, the bone marrow biopsy showed a minimal compromise of CD. A last peripheral blood stem cell collection was tried using high doses of cyclophosphamide but it failed again. After Cyclophosphamide, bone marrow biopsy resulted not compromised by CD. Real-Time PCR for HHV8 DNA was repeat showing persistent viral load. We decided to start oral valganciclovir at a dose of 450 mg twice a day and after two months the treatment led to a clearance of HHV8 DNA from plasma, a complete remission of systemic symptoms and complete remission evaluated by TB-CT and bone marrow biopsy. After three months oral valganciclovir was reduced to 450 mgc twice a day. After nine months the patient is in good health with no evidence of MCD recurrence and reduction of cutaneous Kaposi sarcoma lesions. We described a successful treatment with sequential chemo and antiviral therapy in HHV8 patient: prospective trials are needed in order to establish the best treatment for this rare disease.

**PO-160**

**LOW-DOSE ALEMTUZUMAB AS TREATMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA IN PRETREATED B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA**

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Progressive B-cell chronic lymphocytic leukemia (CLL) is often complicated by autoimmune hemolitic anemia (AIHA), which in same cases may be refractory to conventional therapy. Recently there are same reports who showed that alemtuzumab is an effective agent in refractory AIHA. The aim of our study is to test the efficacy of low dose alemtuzumab in CLL-related AIHA patients. Three male patients had developed AIHA prior the initiation of alemtuzumab therapy. AIHA developed at a median of 51 months from B-CLL diagnosis and 3 months from the last therapy. Previous treatments for B-CLL included CHOP, CVP, chlorambucil, prednisone, fludarabine plus cyclophosphamide, splenectomy, high-dose of cyclophosphamide and autologous peripheral blood stem cell transplantation. All patients received red blood cell transfusions before alemtuzumab therapy, and one of them received an additional transfusion during the treatment. The median haemoglobin value at first alemtuzumab administration was 9.4 g/dl. Alemtuzumab was given subcutaneously at the target dose of 10 mg three times weekly for 30 administrations. AIHA response was defined as the independence from RBC transfusions and a comcomitant > 2.0 g/dl rise in Hb concen-
tion. All three patients responded to alemtuzumab treatment with a >2 g/dL rise in Hb concentration after a median of 8 weeks. One patient responded at week 11, but in this patient treatment had been discontinued for 3 weeks (from 6th to 8th week) because of CMV reactivation. Two patients showed CMV reactivation at the 5th and 6th week of therapy and were treated with oral ganciclovir for 14 and 21 days respectively. The therapy was well tolerated, No episode of febrile neutropenia or bacterial/fungal infection occurred during the treatment. The median total dose of alemtuzumab required to obtain the AIHA response was 220 mg. The median duration of the response was 10 months and only one patient experienced a new episode of AIHA after 26 months. At the end of alemtuzumab treatment the median Hb concentration was 12.7 g/dL. All three patients underwent further treatment because of disease progression after 9, 10 and 26 months from the end of alemtuzumab therapy. Our study demonstrates that AIHA response can be obtained with low dose of subcutaneous alemtuzumab with low hematological complications. Alemtuzumab may be worthwhile to consider before rituximab if AIHA is accompanied by progressive CLL in need of cytoreductive therapy.

PO-161

LOW DOSES OF ORAL FLUDARABINE IN PREVIOUSLY UNTREATED ELDERLY PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKAEMIA: EXPERIENCE OF A SINGLE UNIT


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Background. Oral fludarabine has been demonstrated clinically effective and safe in patients affected by chronic lymphocytic leukaemia (CLL). However, mainly in elderly patients, a high incidence of haematological toxicity and infectious complications have been reported. Aim. we have tested efficacy and safety of oral fludarabine at low doses in previously untreated elderly patients. Patients and Methods. patients received 10 mg tablets of oral fludarabine to a dose of 25 mg/m²/d for 5 days, repeated every 4 weeks, for a total of six cycles. Efficacy was assessed using International Workshop of Chronic Lymphocytic Leukaemia (IWCLL) for response. Safety monitoring included WHO toxicity grading for adverse events. Results. A total of 16 patients received treatment and all patients completed 6 cycles. The median age was 72 years (range 65-82 years). Moreover, 13 out of 16 patients were affected by other chronic disease as diabetes mellitus, essential hypertension, heart failure, or chronic bronchitis. 14 out of 16 patients were evaluable, according to IWCLL criteria. 10 out of 14 patients were responsive to the treatment (3 complete remission and 7 partial remission; ORR 75%), while the remaining 4 patients did not show any response (2 were considered resistant with progressive disease and 2 with stable disease). Median time to progression was 521 days (range 53-701 days). Haematological toxicity was very low in the majority of patients (grade 1-2 neutropenia in 5 out of 16 cases) and did not require treatment. No one patients showed decreased counts of CD4 lymphocytes under 200/microlitre. Moreover there was a significant improvement of haemoglobin value (median 0.8 g/dL, range 0.5-1.2). Conclusions. Oral fludarabine at low doses is highly effective in the elderly previously untreated patients affected by CLL. It induces good response in about 75% of patients. In addition, toxic side effects were negligible and very mild in elderly patients. Moreover, importantly, oral fludarabine can be taken at home, with a greatly reduced administration costs and less time spent in hospital.

PO-162

A COMPARISON OF FLOW CYTOMETRY, BONE MARROW BIOPSY AND BONE MARROW ASPIRATE IN THE DETECTION OF LYMPHOID INFECTION IN B-CLL

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The role of flow cytometric (FC) immunophenotypic analysis in B CLL to detect bone marrow (BM) involvement in clinical staging and to evaluate the effectiveness of treatments has not yet been determined. The aim of this study was to evaluate the diagnostic value of FC in the assessment of bone marrow infiltration in CLL. 17 patients were included in this study (12 men and 5 women; mean age was 72 years; range, 59-81 years). 12 patients were evaluated as part of the initial disease presentation requiring a diagnostic biopsy procedure, the remaining 5 patients were evaluated for restaging of disease while in complete or partial remission or for relapsed disease. In all patients BM aspiration, BM biopsy and immunophenotyping by FC in the aspirate and in PB were carried out simultaneously. In addition to morphological evaluation, immunohistochemistry was performed. Cytogenetic analysis was available only for 6 patients. The comparison between BM aspirates and PB results obtained in FC showed a 100% concordance rate for the typical antigen pattern of B CLL (CD19+, CD23+, CD20+) and for lambda and 73% for k immunoglobulin light chains; also for unfavourable prognostic markers CD38 and ZAP 70 we found a good correspondence: 95% and 77% respectively. ZAP 70 expression analysis was introduced recently in our study so only 9/17 BM aspirates and 11/17 PB samples were tested. All 17 cases (100%) showed concordance between histologic examination and FC (BM+/FC+) in particular a 100% concordance for CD19, CD23, CD20 and 95% for CD22. Study of ZAP-70 expression in FC and immunohistochemistry produced discordant findings. We could not correlate immunophenotype and cytogenetics. In our experience immunophenotypic analysis by FC was a useful tool in the diagnosis of CLL. In particular, a positive PB immunophenotyping in early stage disease correlates with BM involvement (morphology, immunohistochemistry, FC) so a FC analysis of PB could be a sufficient test at diagnosis. In fact our prognostic markers CD38, ZAP 70 and RITUXIMAB evaluation of BM biopsy remains the standard method to evaluate BM involvement and BM infiltration pattern is considered a major prognostic factor but it has been suggested that a lymphoid infiltration ≥ 80% could be more useful than a diffuse infiltration to predict outcome in early stage CLL. FC analysis of BM aspirates might be slightly more sensitive than BM biopsy in detecting minimal residual disease and could be introduced in the follow up of CLL.
behavior. The results obtained with systemic chemotherapy plus rituximab in our case, and in general in large B cell lymphoma, induce us to retain that in aggressive lymphomas it could represent the 1st therapeutic choice.

**PO-164**

**COMPARATIVE STUDY BETWEEN CD5+ AND CD5− CLONAL LYMPHOCYTOSIS: PRELIMINARY DATA**

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Thirteen patients (10 M, 3 F) with CD5−, B-CLL-like chronic lymphoproliferative diseases have thus far been studied. First, lymphocytes were stained with a large panel of monoclonal antibodies in an attempt to identify a peculiar surface phenotype common to all patients. Specifically, fluorochrome-conjugated antibodies specific for the following membrane proteins, including activation, adhesion, costimulatory molecules and growth factor receptors, were used for flow cytometry analysis of CD19, CD20, CD26, CD29, CD38, CD40, CD41a, CD5, CD5, CD70, CD71, CD80, CD86, CD10, CD23, CD5, CD152 (CTLA-4), CD41a, CD51, CD52, CD62L, CD62E, CD62D, CD62C (CRTH2), CD38, CD25, CD103, CD61, CD20, CD89, CD40, CD267 (TACI), CD268 (BAFF-R), CD34, and CD62E. In addition, the percentage of T lymphocytes and the CD4/CD8 ratio were assessed as well. Secondly, because of clinical resemblance to B-CLL, intracellular staining for CD5 was carried out in a number of patients in order to explore the possibility that the CD5− B cells in these undefined chronic lymphoproliferative diseases were actually canonical CLL B cells failing to express CD5 on the cell membrane; furthermore, cells from a number of patients were also activated in vitro with different stimulators (PMA + ionomycin, IFN-gamma, soluble CD40L) to test the possibility of activation-induced CD5 expression. Finally, the surface phenotype of clonal B cells was compared to that of classic B-CLL, as these patients were shown to have a clinical course resembling that of B-CLL patients, to look for similarities and differences between these two lymphoproliferative diseases.

**Results.** Apart from CD19 and CD20, B cells from the CD5−, B-CLL-like patient population were typically positive for CD20, CD27, CD25, CD24, and BAFF-R (median >90% for all surface markers), whereas they were negative for CD10, CD105, CD34 and CD62. A wide variability was seen with the other surface markers examined. When compared to CLL B cells, apart from CD5, a significant difference was observed with CD23 (p=0.01), CD29 (p=0.01), CD51 (p=0.01), and T lymphocyte percentages (p=0.0007). Specifically, canonical CLL B cells appeared to have been recently activated, as suggested by higher CD69 expression rates, and to have a reduced expression of the costimulatory molecules CD80 and CD86. Moreover, T cell percentages were more depressed in classic B-CLL (median, 16% vs 28%), whereas no differences were seen in CD4/CD8 ratios. CD23, although present on all cells in about one third of patients with CD5− leukemia (4 in this series), was globally less expressed when compared to typical CLL B cells. Intracellular protein staining failed to detect cytoplasmic CD5, both in fresh and fixed cells, in about one third of patients with CD5− leukemia (4 in this series), was globally less expressed when compared to typical CLL B cells. Intracellular protein staining failed to detect cytoplasmic CD5, both in fresh and fixed cells, in about one third of patients with CD5− leukemia (4 in this series), was globally less expressed when compared to typical CLL B cells. Intracellular protein staining failed to detect cytoplasmic CD5, both in fresh and fixed cells, in about one third of patients with CD5− leukemia (4 in this series), was globally less expressed when compared to typical CLL B cells. In addition, the percentage of T lymphocytes and the CD4/CD8 ratio were assessed as well.

**Conclusions.** No peculiar set of surface molecules could be identified in CD5− leukemia with the panel of monoclonal antibodies used in this study so as to suggest diagnostic criteria for flow cytometric analysis. Indeed, the surface markers constantly found expressed on CD5− leukemia cells were also found on CLL B cells and are shared by other B-cell chronic lymphoproliferative diseases. When compared to classic B-CLL, differences were seen in the expression of only a few surface markers; finally, leukemia counts appeared to be generally less striking than in B-CLL, with preservation of a higher fraction of T cells. Further studies are ongoing to better dissect homologies and differences between CD5− and CD5+ chronic lymphoproliferative diseases.

**PO-165**

**THE MUTATIONAL STATUS OF THE IMMUNOGLOBULIN VARIABLE REGION GENES (IGVH) IS ABLE TO PREDICT RESPONSE TO THERAPY IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) IN ADVANCED STAGE OF DISEASE TREATED WITH CHLORAMBUCIL (CLB): PRELIMINARY RESULTS**


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Aim of this study was to evaluate the impact of the new prognostic markers: mutational status of IGVH region genes, chromosome analysis by FISH and expression of CD38 and ZAP70 on leukemic cells, on response to therapy of B-CLL patients in advanced stage of disease treated with Chlorambucil. Materials and Methods. Twenty-seven B-CLL patient, 18 males with a mean age of 67.3 (SD±10.1), 5 in Rai stage 0, 18 I/II and 4 III/IV, 8 in Binet stage A, 15 B and 4 C, have been enrolled in this study. Unfavorable prognostic factors were considered expression on leukemic cells of CD38>30%, ZAP70 strongly at Western Blot analysis, IGVH unmutated and detection at cytogenetic analysis of 17p- and 11q- deletions. Criteria for treatment were stage III-IV/C and in the other stages the presence of active disease signs. All patients received CLB administered at standard monthly dose of 10 mg/day X 14 days for 6 courses. Response to therapy was defined as follows: complete response (CR) as the absence of adenopathies and splenomegaly at physical and radiologic examinations, Hb > 11g/dl, platelet count >100×10^9/L, peripheral lymphocytes <4×10^9/L and PMN > 1.5×10^9/L, partial response (PR) as the reduction > 50% of the original enlargement of spleen, adenopathies and/or peripheral lymphocytosis; no response (NR) as a decrease < 50% of the above features. Results. Comparing the clinical stages with the new prognostic markers, a significant relationship has been found between Rai stage and IGVH mutational status. (see table)

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<th>RAI</th>
<th>Unmutated</th>
<th>Mutated</th>
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<tr>
<td>0</td>
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<tr>
<td>I/II</td>
<td>15</td>
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<td>18</td>
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<td>III/IV</td>
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<td>Total</td>
<td>19</td>
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According to the response criteria, 16/27 (59.2%) of patients obtained a CR, 8/27 (29.6%) a PR and 3/27(11.1%) a NR. Comparing all the prognostic variables with response, only the IGVH mutational was able to predict the clinical response. Interestingly, all the 8 mutated cases reached CR, while in the 19 IGVH unmutated patients, 8 obtained a CR, 8 a PR and 3 a NR (p=0.02). Conclusion. Patients with a mutated IGVH status seem to benefit of CLB treatment, because they have a high probability to obtain CR.
PO-166
ORAL FLUDARABINE IN R-FC REGIMEN IN THE TREATMENT OF CHRONIC LYMPHOCYTIC LEUKAEMIA
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Introduction. Rituximab plus Fludarabine and Cyclophosphamide (R-FC) is the best treatment of Chronic Lymphocytic Leukemia (CALL) available up to today. The original schedule with intravenous Fludarabine is difficult to be applied in outpatients setting. Therefore we tested the oral formulation of Fludarabine scheduled at 40 mg/sqm associated with Cyclophosphamide and Rituximab in a series of 18 outpatients affected by CALL. Methods. from June 2004 to September 2006 eighteen patients were treated in the Hematology Department in Cagliari, Italy. Informed consent was obtained by all patients before start of therapy. 13 patients were males and 5 females; median age was 59,5 years (range 34-71). All patients were B or C Binet stage in progression (11 B, 7 C), all but one with no B symptoms. All patients presented CALL standard diagnostic criteria, 8 pts were untreated and receiver therapy as first line treatment. Ten patients were in second or subsequent line of treatment (range 2-6); 9 patients had received Fludarabine containing regimens. Chemotherapy schedule consisted of Rituximab 375 mg/sqm iv on day 0 followed by Fludarabine 40 mg/sqm day and Cyclophosphamide 250 mg/sqm day po day 1 to 3, starting next day of Rituximab. Courses were repeated every 28 days for a total of 6 cycles. All patients received anti-infective prophylaxis with Fluconazole and Levofloxacine. HBV positives patients received prophylactic Lamivudine. Results. All patients but one completed planned therapy with no relevant delay for granulocytepe尼亚. Among the eight patients treated as first line therapy 6 achieved Complete Remission and 2 a Very Good Partial Remission. There were no toxic death but life threatening infections (all patients received Filgrastim). About patient treated as second or subsequent line of therapy six achieved Complete Remission, Four a Partial Remission received therapy. No toxic death but four patients shows systemic infections requiring hospitalization. The median follow up was 20,5 months (3-32). Relapse or progression of disease was seen in 2 first line patients (after 9 and 19 months, respectively); in the pretreated group there were 4 relapses (range 6-13 months). All patients are still alive. Conclusions. The association of intravenous Rituximab, oral Cyclophosphamide and oral Fludarabine is an effective and feasible therapy with excellent therapeutic patient's compliance. The advantage of an outpatient schedule must be confirmed by further controlled studies and longer follow-up.

PO-167
INITIAL (LATENT) STAGE OF POLYCYTHAEMIA VERA WITH THROMBOCYTOPAECY. THE IMPACT OF THE BONE MARROW BIOPSY AND JAK2V617F MUTATION FOR THE DIFFERENTIAL DIAGNOSIS WITH ESSENTIAL THROMBOCYTHAEMIA.
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Polythmaemia Vera (PV) is a chronic myeloproliferative disorder (CMPD) characterized by increased erythropoiesis, granulopoiesis and megakaryocytes. According to the WHO classification, two stages of PV are recognizable, known as polycythemic phase and post-polycythemic myelofibrosis. It has been suggested that PV could be preceded by an early latent phase, in which the increase in RBC mass or Hb are lower than requested for the diagnosis by the diagnostic criteria of the Polycythemia Vera Study Group and the WHO classification. The European Clinical and Pathological (ECP) criteria for diagnosis of early PV have been recently published. The acquired activating mutation of JAK2 tyrosine kinase, JAK2V617F, is present in granulocytes of about 97% of PV patients, 52-57% of ET, 45-50% of agnogenic myeloid metaplasia and, less frequently, in other CMPDs. On the contrary, it has not been found in normal controls or secondary erythrocytosis, carrying a positive-predictive value in distinguishing CMPDs from non clonal conditions such as secondary erythrocytosis. In this study we examined the clinical features, bone marrow biopsies and JAK2V617F mutational status of 17 ePV patients, who presented at diagnosis the clinical and morphological features of ET, and manifested a well-developed polycythaemic phase of PV during the follow-up (median 8.6 yrs; range 2-17 yrs), compared with 19 PV and 14 ET cases (according to WHO) as controls. ePV patients revealed at diagnosis increased Hb (ePV:15.5 g/dl; ET:13.8 g/dl; PV:16.9 g/dl), Hct (ePV:45.9%; ET:41%; PV:51.8%) and Plts (ePV:854 × 109/L; ET:877 × 109/L; PV:691 × 109/L), splenomegaly (ePV:43%; ET:0%; PV:61%) and hepatomegaly (ePV:33%; ET:14%; PV:61%). Morphological examination in ePV patients demonstrated moderate to marked increase of BM cellularity (ePV:65%; ET:0%; PV:73%) and pleomorphic megakaryocytes (ePV:23%; ET:20%; PV:100%). Increased (ePV:100%; ET:14%; PV:100%) and left-shifted erythropoiesis (ePV:32%; ET:0%; PV:79%), and increased (ePV:65%; ET:14%; PV:100%) and left-shifted granulopoiesis (ePV:65%; ET:0%; PV:58%) were also found. 15/15 ePV cases carried the JAK2V617F mutation (6 homo, 9 hetero), in comparison to 7/13 ET (1 homo, 6 hetero) and to 13/19 PV (5 homo, 13 hetero). In conclusion, these results confirm the existence of a early phase of PV that may mimic ET. A diagnostic algorithm, useful to differentiate ePV from ET, may be obtained by the clinical, morphological and molecular features of each patient.

PO-168
INFLUENCE OF JAK2V617F ALLELE BURDEN ON PHENOTYPE IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA
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Background. The correlation of JAK2V617F mutation with clinical phenotype of Ph-cMPD patients is still under debate, in part because of heterogeneity of available series and of a discontinuous (hetero vs homo) rather than continuous quantitative approach to mutational load. Aim. As an approach to unravel significant associations between phenotype and JAK2 mutation, we have correlated the levels of JAK2V617F allele load with clinical and laboratory characteristics at diagnosis in a series of patients with essential thrombocythemia (ET). Methods 260 patients with ET, diagnosed according to WHO criteria, were studied. Median
age was 52 years (range, 16-93). JAK2V617F allele ratio was evaluated at diagnosis by a Taqman one-step assay on granulocytes DNA. Results. 195 patients (65%) were JAK2V617F mutant, and the percentage of those displaying >51% mutant allele content (homozygous status) was 5%. Mean level of mutant JAK2 allele was 23±17% (range 1-87%), that is significantly lower than the level we previously found in PV (n=185; 54±24%; p<0.001) or in patients with PMF (n=55, 43±42%; p<0.001) or secondary forms of myelofibrosis (n=20, 60±58%; p<0.001). Overall, 64 patients (25%) actually presented JAK2V617F levels greater than 25%, while only 3 patients (1%) had levels greater than 75%. JAK2V617F mutant patients had significantly higher leukocyte count (p=0.02), hemo-globin (p<0.0001) and Htc levels (p<0.0001), ALP level (p=0.04) and lower platelet count (p=0.05), confirming previous observations. However there was no clear dose-dependent correlation between these haemato-logic parameters (at diagnosis or at the time of blood sampling) and the load of mutant allele. Among JAK2V617F mutant patients, age at diagnosis was significantly correlated with the load of JAK2V617F allele (p<0.0001, CI 95% 0.16-0.44). The frequency of patients presenting splenomegaly progressively increased according to JAK2V617F allele burden (p=0.02 2 test); furthermore, there was a significant correlation between JAK2V617F ratio and presence of spleen size larger than 15 cm (p=0.02, 2 test). Mutant allele burden had no impact on puritus, systemic symptoms, need of chemotherapy and duration of disease. Thrombotic complications were referred by 17% of patients and 24% of JAK2V617F mutant patient, respectively (p=0.3); however we found that there was a trend towards more frequent thrombotic events in patients with higher levels of JAK2V617F allele load (p=0.08 χ2 test). On the other hand we observed a significant correlation between EEC and the percent-age load of JAK2 mutant allele while, notwithstanding FRV-1 expression levels were significantly higher in mutant than in wild type patients (p=0.53), there was no overt correlation with the JAK2V617F allele burden. Conclusions. These data indicate that in ET patients laboratory abnormalities and defined aspects of clinical phenotype are only partially related to the relative representation of V617F allele in the context of normal hematopoiesis; this might be due to the low allele bur-den and/or other, but still poorly characterized, gene or host modifiers.

PO-169
LONG-TERM EVALUATION OF 404 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: CLINICAL OUTCOME, EFFICACY AND SAFETY WITH RESPECT TO DIFFERENT THERAPIES
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Essential Thrombocythemia (ET) is a myeloproliferative disorder associated with persistent thrombocytosis. The main clinical features are recurrent thrombotic and/or hemorrhagic events involving both the arterial and venous systems. Data concerning the role of platelet-lowering agents to prevent thrombotic and hemorrhagic events are not conclusive. We retrospectively analyzed 404 ET patients (pts) referred to our Institute between January 1977 and December 2004, with a median follow-up of 84 months (1-318). Median age at diagnosis was 64 (15-91) years; median platelet (PLT) number was 787(465-3700)x10^9/L. Three hundred and sixty-two out of 404 (89.6%) pts were treated with platelet-lowering agents: Hydroxurea (HU;135 pts), Busulphan (BU;104 pts), HU+BU (66 pts), Interferon-alpha (IFN-alpha12 pts), HU+IFN-alpha (21 pts), BU+IFN-alpha (6 pts) or HU+BU+IFN-alpha (6 pts). Two hundred and ninety-six pts were treated because of high risk and 66 pts for either cardiovascular risk or symptoms associated to microvesSEL disturbance. In 71/362 (19.6%) and 237/362 (65.5%) pts the treatments were able to maintain the PLT number for at least 2/3 of their follow-up, <400x10^9/L (Complete remission) and 400-600x10^9/L (Partial remission), respectively. Only 14 pts were withdrawn from HU or BU for toxicity. A total of 365 (91.1%) pts received antiplatelet agents (233 Aspirin, 65 Ticlopidine, 70 others) for a median period of 53 (1-252) months; this therapy was stopped in 87 pts, in most cases after achieving the control of platelet count (<600x10^9/L). During follow-up, we registered 61 thrombotic events in 44 (10.9%) pts, and 37 hemorrhagic events in 32 (7.9%) pts, while diizzi-ness, headache and other symptoms due to microvascular disturbance were not different from those of patients treated with single drugs. Patients treated with HU and BU developed a significantly higher number of thrombotic events compared to pts who received a single drug (p=0.004). Previous thrombotic event, age and platelet number at diagnosis, thrombocytosis control during follow-up and presence of one or more common cardiovascular risk factors were evaluated in order to establish any correlation with thrombotic risk. Only the first two factors were significantly correlated. Blastic transforma-tions, myelofibrosis evolution and solid malignancy occurred in 3(0.7%), 15(3.7%) and 21(5.2%) pts, respectively. The three pts who showed blastic transformation were treated with a particularly high total dose of HU (3.089.500 mg), if compared to the median dose administered (462.000 mg) in one case or with the sequential association of HU and BU in the other two cases. The overall survival (expressed by the Kaplan-Meier curve) was approximately 85% at 12 years after diagnosis, which is similar to the life expectancy of the normal population. In conclusion, in our experience HU and BU resulted effective in controlling thrombo-cytosis in ET pts with negligible toxicity. Previous thrombosis and older age at diagnosis significantly increase the risk of thrombotic events during follow-up. The blastic transformation seems to be related to the HU+BU association or to higher total HU dose administered.

PO-170
FAMILIAL CHRONIC MYELOPROLIFERATIVE DISORDERS: CLINICAL PHENOTYPE AND EVIDENCE OF DISEASE ANTICIPATION
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Introduction. Chronic myeloproliferative disorders (CMD) have a sporadic occurrence, however familial clustering was reported. To assess the prevalence and the clinical phenotype of familial CMD, to study the anticipation of disease onset in successive generations. Methods. An interview-based investigation of family history was performed to identify familial cases among 458 patients with apparently sporadic CMD. Clinical phenotype of familial CMD was compared to that of sporadic CMD. Anticipation was studied evaluating both age at diagnosis and telomere length (flow-FISH) in successive generations. Results. Among 458 patients with sporadic CMD, the prevalence of familial cases was 7% (54 pedigrees; 75 patients). Kolmogorov-Smirnov and two tailed Fisher exact tests did not demonstrate any significant difference in clinical presenta-tion between patients with familial and those with sporadic CMD. Within 544 person-years of follow-up, patients with familial CMD developed complications and disease-evolutions as like as those of sporadic CMD. Comparing second-generation to first-generation patients, Wilcoxon Matched Pair test demonstrated a significantly lower age at diagnosis of second-generation patients (p<0.001), and Nelson-Aalen estimator showed a significantly higher age-dependent hazard of CMD onset in second-generation patients (p<0.001). We observed a significant telomere shortening in offspring compared to parent (p<0.043). Dis- cussion. This study suggests a thorough investigation of family history in the management of patients with CMD. Clinical phenotype of patients with familial CMD is similar to the phenotype of sporadic CMD. The age distribution between parent and offspring and the kinet-ic of telomere shortening provide evidence of disease anticipation in familial CMD.

PO-171
CLINICAL AND PROGNOSTIC IMPLICATIONS OF BONE MARROW FIBROSIS GRADING IN CHRONIC IDIOPATHIC MYELOFIBROSIS (CIMF)
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CIMF is a Philadelphia chromosome-negative chronic myeloprolifera-tive disorder that arises from a pluripotent hematopoietic progenitor cell with the deposition of fibrin and collagen in bone marrow via angiogenic factors. Its clinical presentation is characterised by progressive anemia, leukocytosis or leukaemia, circulating blasts, constitutional symptoms, extra-medullary hematopoiesis and splenomegaly. Expected survival is 3-
10 years, and transformation to acute leukemia may occur. The aim of this study was to determine whether the European consensus parameters for grading bone marrow fibrosis in CIPM have clinical and/or prognostic implications. The study population included 113 consecutive CIPM patients (61 males and 52 females with a median age of 67 years, range 27–86) diagnosed according to the Polycythemia Vera Study Group (PVSG) criteria between 1996 and 2006, and followed up for a median of 20 months (range 4–126). On the basis of the WHO criteria, their bone marrow biopsies were classified as indicating CIPM (95 pts) and post-poly- cythemia vera PMF (5 pts), whereas the European consensus parameters identified 38 patients with CIPM-0, 27 with CIPM-1, 26 with CIPM-2, and 12 with CIPM-3. Hemoglobin (Hb) levels at diagnosis were higher in the CIPM-0 vs all the other groups (p<0.0017). Platelet (PLT) levels were higher in CIPM-0 vs all the other groups (p=0.0081), and also in CIPM-1 vs CIPM-3 (p=0.00009) and post-PVF MF (p=0.027), and in CIPM-2 vs CIPM-3 (p=0.007). Lactate dehydrogenase (LDH) levels were lower in CIPM-0 vs all the other groups (p=0.03), and also in CIPM-1 and CIPM-2 vs post-PVF MF (p=0.006). The spleen was progressively enlarged from CIPM-0 to post-PVF MF (p=0.013). Median survival estimated by Kaplan-Meyer analysis was significantly different between the groups (CIPM-0, CIPM-1, CIPM-2, CIPM-3; post-PVF MF: log-rank test p=0.01), progressively worsening from CIPM-0 through CIPM-3 with post-PVF MF presenting a survival similar to CIPM-3. Genetic lesions, circulating blasts and secondary tumours occurred significantly more often in CIPM-0 vs the other cases with histological grade of fibrosis according to post-PVF MF. No differences in the frequency of thrombotic/hemorrhagic events or erythromelalgia/paresthesia. In conclusion, bone marrow fibrosis grading shows clinical correlations and prognostic implications in CIPM.

PO-172 HYPOMETHYLATING AGENTS AND HDAC INHIBITORS EFFECTS ON THE CXCR4 EXPRESSION IN CD34+ CELLS OF PRIMARY MYELOFIBROSIS

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Introduction. Primary Myelofibrosis (PMF) is characterized by increase of CD34+ hematopoietic stem/progenitor cells in the peripheral blood (PB), but the mechanisms implicated are not completely identified. Evidence supported an alternative mechanism by which down-regulation of CXCR4 and disruption of the CXCR4/SDF-1 axis may directly act on HPC mobilization. The aim was to characterize the possible role of CXCR4 abnormalities in the constitutive CD34+ cell mobilization. Methods. CD34+ cells were isolated from PMF PB, and subjected to FACS analysis, in vivo expression of mRNA was quantified by RT-PCR. Results. As supported by previous study we observed that CXCR4 is expressed at lower levels on the membrane of circulating CD34+ cells in PMF as the consequence of reduced gene transcriptional activity. Furthermore, CXCR4 RNA were strictly correlated with the number of circulating HSC. To address potential mechanisms involved in the down-regulation of CXCR4 RNA, we first evaluated the effects of CD34+ cell exposure to a range of hypomethylating agents and histone deacetylase inhibitors which all have been supposed to play some role in PMF pathogenesis and/or in CD34+ mobilization. We found that only DFP-1 was able to down-regulate the membrane content of CXCR4, while mRNA levels were unchanged, to suggest ligand-induced receptor internalization but not effect on gene transcription. We hypothesized that epigenetic modifications might be involved in down-regulation of CXCR4. HCL cells, as well as the CD34+ purified from 10 PMF patients, were treated with 5-azacytidine (5-AZA) and SAHA. Among both these models, we observed significant up-regulation of CXCR4 over the levels obtained with 5-AZA only. The increase of CXCR4 could be expression on the membrane; the combination of 5-AZA and SAHA determined only minor increase detected as soon as 8–12h. There was a discrete methylation pattern of CpG islands involving arterial vessels in 6 cases and venous vessels in 2. Hence, they were investigated by allele-specific PCR using DNA from peripheral blood samples according to Scott et al. (N Engl J Med 2007; 356:459) for the presence of the following JAK2 exon 12 mutations: F537-K589delinsL, H588QK589L, K589L, and N542-E543del. Results. No patient carried the F537-K589delinsL, H588QK589L, or K589L mutation. The N542-E543del mutation was detected in 3 patients (11.1% of the whole JAK2 V617F-negative PMF patients, 2.7% of the whole JAK2 V617F-negative PV patients). In the first patient was a 61 y.o. man with splenomegaly and a history of proximal deep venous thrombosis of the leg; Hb was 19.2 g/dL, Htc 0.60; erythropoietin (Epo) was 1.5 U/L (normal range 2-19). The second patient was a 49 y.o. woman with splenomegaly and a history of ischemic stroke; Hb was 17.7 g/dL, Htc 0.53; Epo was 0.1 U/L. The third patient was a 17 y.o. man asymptomatic; Hb was 17.0 g/dL, Htc 0.54; Epo was 3.2 U/L. In this latter patient the growth of erythroid colonies was significantly dependent of Epo. Conclusion. In PV patients a significant portion of individuals JAK2 V617F-negative carried the N542-E543del mutation in the exon 12. We were unable to confirm the presence of other mutations in our sample. Thus the overall prevalence of exon 12 mutations among the JAK2 V617F-negative PMF patients is 11%. This low proportion of JAK2 exon 12 mutations other than V617F in comparison to the aforementioned study of Scott et al. could be explained by the source of DNA (peripheral-blood granulocytes instead of bone marrow cells); investigations on bone marrow samples from the remaining negative patients are underway to improve the diagnostic yield.

PO-174 RESPONSE TO DASATINIB IN PATIENTS WITH AGGRESSIVE SYSTEMIC MASTOCYTOSIS WITH D816V KIT MUTATION

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Human systemic mastocytosis (SM) is a rare disease caused by an
an abnormal mast cell accumulation in various tissues. It usually occurs as a sporadic disease that is often persistent or progressive in adults. Clinical course is variable. The most aggressive forms have a rapid course and required treatments to reduce the neoplastic burden and to slacken the progression. Unfortunately no therapy have been demonstrated efficacy, mostly in the treatment of aggressive systemic mastocytosis (ASM). SM has been associated with constitutive activating c-kit somatic mutations, the most frequent of whom is the D816V mutation. Kinase inhibitors blocking constitutive, such as imatinib, have been used in SM, but they had no effect on D816V mutant kit. Here, we report on six patients with Systemic mastocytosis and with detectable D816V mutation treated with dasatinib 70 mg BID as in leukemia treatment. According to the WHO Classification of Systemic mastocytosis, we describe clinical characteristics of six cases of ASM with different organ damage treated with dasatinib for various period, and we made evaluation of response according to Valant criteria (Leuk Research, 2005). All patients excepting Patient 3 reached the dose of 140 mg daily. Patient 1, 2 and 4 are on treatment after 9, 8 and 6 months respectively, without any signs or symptoms of disease progression. Patient 3 and Patient 5 interrupted dasatinib for worsening clinical condition after 40 days and three months respectively. Patient 6 stopped dasatinib after five months for progression to acute leukemia. All patients had to suspend therapy due to extra-haematological toxicity. The most commonly toxicities were on gastroenteric tract, with diarrhoea and abdominal pain, hypotension and pleural effusion. Symptoms resolved or return to mild grade with dose reduction. We noticed major response in three patients for the prompt resolution of signs of organ damage recorded during dasatinib treatment, and we also attained a measurable decrease of the burden of neoplastic MCs by means of trypaflavine level monitoring in all but one patients. Acknowledgments: COFIN 2008 (Molecular therapy of Ph- leukemias), by PBII 2001, by the University of Bologna (60%), by the Italian Association for Cancer Research (A.I.R.C.), by the Italian National Research Council (C.N.R.), by Fondazione Del Monte of Bologna and Ravenna (Italy) and A.I.L. grants, LeukemiaNet grants.

PO-175
GENETIC AND EPIGENETIC INACTIVATION OF SOCS-1, SOCS-3 AND SHP-1 IN PH-NEGATIVE MYELOPROLIFERATIVE DISORDERS

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Introduction. Ph-negative chronic myeloproliferative disorders (CMPD) are characterized by somatic point mutations of the JAK2 gene, leading to constitutive JAK/STAT activation. The negative regulators of cytokine signaling SOCS-1, SOCS-3 and SHP-1 have a crucial function in the down-regulation of JAK/STAT pathway. SOCS-1, SOCS-3 and SHP-1 may be silenced by aberrant DNA methylation and/or mutation in human malignancies and in familial cases [1]. Methylation and genetic inactivation of SOCS-1, SOCS-3 and SHP-1 occur in: i) 112 CMPD, including 43 essential thrombocytopenia (ET), 28 polycythemia vera (PV), 121

also when the analysis was restricted to PV, ET and MF each as a single group and after stratification for JAK2V617F mutation. The correlation between hypermethylation and gene expression was analyzed by real-time PCR. SOCS-3 mRNA levels were significantly higher in unmethylated samples compared to methylated samples (p<0.005) and to normal bone marrow (p=0.03). Similar results were obtained for SOCS-1. SOCS-1 and SOCS-3 missence mutations were detected in 2/104 (1.9%) and 1/93 (1%) CMPD, respectively. Discussion. i) Inactivation by aberrant methylation of SOCS-3, SOCS-1 and SHP-1 is involved in the pathogenesis of CMPD, is selectively associated with neoplastic hemopoiesis, and correlates with reduced gene expression; ii) methylation of SOCS-3, SOCS-1 and SHP-1 occurs in both JAK2V617F positive and negative cases; iii) the methylation rate of SOCS-3, SOCS-1, and SHP-1 is similar in CMPD and in AML post-CMPD, suggesting that SOCS-3, SOCS-1 and SHP-1 silencing is not involved in leukemic transformation; iv) SOCS-1 and SOCS-3 mutations are rarely involved in CMPD.

PO-176
PLATELET, GRANULOCYTE AND PLASMA HEMOSTATIC MOLECULES IN PATIENTS WITH ESSENTIAL THROMBOCYTHESMAH (ET) WITH AND WITHOUT THE V617F JAK-2 GENE MUTATION

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Introduction. An acquired gain-of-function mutation (V617F) in the tyrosine kinase JAK2 gene is detected in neutrophils and platelets from about 50% of patients with ET. Clinical data indicate an association between this mutation and the severity of the disease. We wanted to evaluate in a large number of subjects whether the presence of the V617F JAK2 mutation identifies ET patients with specific hemostatic abnormalities. Methods. Ninety three consecutive ET patients (F/M= 53/40; age range = 17-86), 44 V617F JAK2 carriers and 49 JAK2 wild-type (wt), and 50 control subjects, were enrolled into the study. Platelet surface molecules, platelet-polymorphonuclear leukocyte (PMN) aggregates, and PMN surface activation molecules, were analysed by flow cytometry. The plasma levels of hypercoagulation markers [F1+2, TAT complex, D-Dimer, Elastase and Thrombomodulin (TM)] were measured by ELISA. Results. Analysis of platelet surface from the overall ET group, showed higher tissue factor (TF) and P-selectin (p<0.01), and lower CD41 and CD42b (p<0.01), compared to healthy controls. The V617F JAK2 carriers had significantly (p<0.01) higher levels of TF-positive platelets than the wt subjects; this was confirmed by measurement of platelet TF antigen levels. Significantly (p<0.05) higher levels of platelet/PMN aggregates were measured in JAK2 mutation carriers compared to wt ET and control subjects. PMN expression of CD14, TF, CD11b and LAP were significantly higher in ET versus controls, with CD14 and LAP being highest in the JAK2 mutation carriers (p<0.05). The levels of hypercoagulation markers were all significantly greater in the plasma of ET patients compared to healthy controls. However, only TM level confirmed to be statistically different between the V617F JAK2 carriers and wt ET (p<0.01). All the difference between the two subgroups of ET patients (i.e. WBC and PMN count, platelet TF, PMN CD14 and LAP, and plasma TM) remained statistically significant after multivariate analysis. Discussion. These data show that the V617F JAK2 mutation in ET influences specific patterns of hemostasis and inflammation. Prospective studies are required to define the role of these hemostatic abnormalities in predicting thrombotic events in ET patients.
PO-177 ATTENUATED ESSENTIAL THROMBOCYTHEMIA INDUCED IN MICE BY THE EXPRESSION OF AML1/MDS1/EVI1 (AME) OR AN AME MUTANT: COMPARISON BETWEEN TWO NEW MODELS OF MYELOPROLIFERATIVE DISORDERS
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Essential thrombocytemia (ET) is a myeloproliferative disorder characterized by a primary increase in platelets count often associated with splenomegaly and/or hepatomegaly and often thrombosis. The t(5;12)(q26;q22) resulting in the AML1/MDS1/EVI1 (AME) fusion gene is associated with several hematological disorders often characterized by severe dysmegakaryopoiesis, suggesting that AME alters the megakaryocytic program. By BM infection and transplantation we generated two groups of mice that express either wild type AME or a point/insert/deletion mutant of AME (AME-D-C). This mutant lacks the eighth zinc finger motif and is unable to interact with the CtBp corepressor. Both groups of mice developed a fatal disease with features of human ET, however, Kaplan-Meier analysis showed a significant difference (p=0.0032) in survival between the two groups with the AME-D-C-mice living considerably longer. The morphological analyses of the peripheral blood smears did not show significant differences between AME- and AME-D-C-mice. However while the AME-mice were anemic, the AME-D-C-mice had normal hemoglobin. In both groups the platelet number was increased, and atypical features like macro-platelets, agranular platelets, and ovoid megakaryocytes were observed. Both groups of mice developed thrombocytosis with median platelet values consistently above normal, whereas neutrophil counts were normal. It is possible that death resulted from bleeding complications, maybe due to an intrinsic platelet dysfunction more evident in the AME population. Peripheral blood smear showed in both groups of mice evidence of megakaryocytic fragments, large cytoplasm pieces containing pro-platelet, and nucleus scraps. Seven out of 12 AME-mice and 8 out of 12 AME-D-C-mice showed slight neutrophil dysplastic features such as hyposegmentation and open nuclei. The red blood cells were normochromic and normocytic in all mice; however, minimal anisocytosis was observed during the course of the disease, with a variation in degree of polychromatophilia at the time of death. The bone core biopsies were normocellular in eight AME mice, hypocellular in three and hypercellular in one. In contrast, the cellularity in all the AME-D-C samples was normal. The most prominent aspect of the AME-mice were increased number of BM megakaryocytes often grouped in clusters of four to six elements located in proximity of sinuses or in paratrabeculae, and rare megakaryocytes containing intact white cells in their cytoplasm. These two features, previously described as endosteal dislocation and emperipolesis, often seen in patients with MPD or ET, were absent in AME-D-C-mice, in which a mutation in the eighth zinc-finger motif of AME could attenuate the myeloproliferative phenotype caused by AME, particularly in terms of histological features and overall survival.

PO-178 HIGHER LEUKOCYTE COUNT AS A RISK FACTOR FOR THROMBOSIS IN ESSENTIAL THROMBOCYTHEMIA
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Background. Thrombotic complications represent one main feature of Philadelphia neg-chronic myeloproliferative disorders (cMPD); advanced age and prior vascular events have been identified as risk factors for thrombosis, and are currently used for stratifying patients into different risk categories. It has been recently suggested that increased leukocyte count at diagnosis in both ET (Carobbio A, Blood Nov2006) and PV patients (Landolfi R, Blood Nov2006) is associated with thrombosis during follow-up. Aim. In order to address this issue, we evaluated retrospectively 705 patients diagnosed with ET according to either PVSG or WHO criteria, in the Hematology Unit in Florence and Vicenza, Italy, in the period from January 1980 to December 2006. Results. There were 473 females (67%). Median age was 59 years (range, 14-96), median platelet count at diagnosis was 847×10^9/L (range, 480-8125) and median leukocyte count was 9.1×10^9/L (range, 3.9-58). Median follow up was 71 months (range, 1-517). Major cardiovascular (CV) events were recorded in 159 patients (22.5%). 94 patients (59%) had thrombosis at diagnosis whereas 65 (41%) had a CV event during follow-up; 14 out of the 94 patients with thrombosis at diagnosis had re-thrombosis during follow-up. JAK2V617F mutation (allele specific PCR) was detected in 204 (57%) of 359 patients evaluated. By dividing patients in classes according to leukocyte count, the percentage of patients with CV increase from 15% with leukocyte count <5 to 17% with leukocyte 5-7.5, to 22% with leukocyte 7.5-10, to 26% with >10-12.5×10^9/L leukocytes (p=0.01, chi square test for trend). Considering as the reference population patients with leukocyte count <7.5×10^9/L, those with leukocyte count above 10×10^9/L had a significantly increased risk of thrombotic events (RR 1.64; 95% CI 1.10 to 2.5; p=0.02). The role of JAK2 mutation was also examined. JAK2V617F mutant patients had significantly higher leukocyte count, hemoglobin and Hct levels, and lower platelet count, confirming previous observations. The percentage of patients with leukocytes above 25% was 75% in JAK2 positive and 19% in the presence or absence of mutation, respectively (p=0.3). Due to the correlation between JAK2V617F and higher leukocyte count, the effect of leukocyte count on CV events in patients stratified according to mutational status was also evaluated. It was found that higher leukocyte count was associated with thrombosis irrespectively of the presence of JAK2V617F allele (Chi-square test for trend, p=0.05 for both). The role of leukocytes was maintained also in multivariate analysis that included as covariates age, hemoglobin, hematocrit, and platelet count (p=0.038). Patients with higher leukocyte count had an increased risk of venous thrombosis-embolism (p=0.03) while no overt effect was seen as concerns arterial events. Conclusions. We conclude that higher leukocyte count constitutes a risk factor for thrombosis in patients with ET, that is independent of patient age and JAK2V617F mutational status. The emerging role of leukocytosis as novel risk factor for CV events might impact on current criteria for patient risk stratification and prompt prospective trials aimed at evaluating the impact of cytotoxic therapy on thrombosis prevention.

PO-179 CLINICAL AND LABORATORY DIFFERENCES AT DIAGNOSIS BETWEEN JAK2V617F POSITIVE AND NEGATIVE PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA: A SINGLE CENTRE EXPERIENCE
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Introduction. Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder (CMD), characterized by bone marrow reactive fibrosis, extramedullary hemopoiesis, progressive anemia and marked splenomegaly. Recently a gain-of-function mutation exchanging valine to phenylalanine at position 617 (V617F) of the Janus Kinase 2 (JAK2) protein has been described in CMD, particularly in 90% of Polycythemia Vera (PV), and about half of patients (pts) with Essential Thrombocythemia and MMM. Methods. We screened 31 alive MMM pts referred in our hematology unit from 1998 to 2006 for JAK2 mutation status, using allele-specific PCR (cfr Baxter, et al., Lancet, 2005), in order to compare clinical and laboratory features at diagnosis between JAK2 positive and negative pts. Comparisons between categorical and continuous variables were performed by chi-squared statistics and by Wilcoxon rank-test, respectively. Results. We identified 25 (74%) positive and 8 (26%) negative JAK2 pts. There was no significant difference in age (69 vs 68 years), sex (M:F ratio 14:9 vs 3:5), and white cell count (WBC 13,090 vs 10,750×10^9/L; p=0.028), and lower PLT count (360 vs 746×10^9/L; p=0.03) while no overt effect was seen as concerns arterial events. Conclusions. We conclude that higher leukocyte count constitutes a risk factor for thrombosis in patients with ET, that is independent of patient age and JAK2V617F mutational status. The emerging role of leukocytosis as novel risk factor for CV events might impact on current criteria for patient risk stratification and prompt prospective trials aimed at evaluating the impact of cytotoxic therapy on thrombosis prevention.
**PO-180**

**POTENT AND SELECTIVE INHIBITION OF EEC COLONY FORMATION IN JAK2V617F POLYCYTHEMIA VERA AND THROMBOCYTHEMIA BY LOW DOSES OF ITF2357, A NEW HISTONE DEACETYLASE INHIBITOR**


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*Introduction.* A somatic point mutation in JAK2 gene (JAK2V617F) has been recognized as the key pathogenetic lesion in Polycythemia Vera (PV) and Essential Thrombocythemia (ET). Histone-Deacetylase inhibitors (HDACi) are known inducers of cell differentiation and apoptosis of neoplastic cells. ITF2357 is a new HDACi (Italfarmaco, Milano, Italy) which, at low micromolar concentration in vitro, inhibits the secretion of several cytokines and exerts a potent anti-tumor activity against multiple myeloma and acute myeloid leukemia cells. Clinical trials are currently ongoing in this setting. We investigated the ability of ITF2357 and the prototypic HDACi Suberoyl Anilide Hydroxamic Acid (SAHA) to inhibit the spontaneous endogenous erythroid colony (EEC) growth of hematopoietic stem cells obtained from patients with PV (all positive for JAK2V617F), ET (JAK2V617F positive in 53%) and Idiopathic Erythrocytosis (all negative for JAK2V617F). Methods. *In vitro* EEC assays was performed using mononuclear cells (MNC) from peripheral blood samples obtained from PV, ET and IE patients. The inhibitory activity played by log scale concentration of ITF2357 (from 0.01 to 0.75µM) with or without the addition of exogenous cytokines. We also investigated the JAK2V617F at the single colony level on colonies picked-up at the end of a 14 days EEC assay. The molecular analysis of JAK2V617F was performed using allele specific Polymerase Chain Reaction on DNA extracted by a single colony. Results. MNC obtained from IE or ET patients negative for JAK2V617F neither exhibited spontaneous EEC formation nor EPO hypersensitivity (from 0.1 UI/mL up to 10UI/mL), while MNC from JAK2V617F PV and ET patients invariably sustained the spontaneous outgrowth of JAK2V617F mutated colonies. ITF2357 induced a 90% EEC inhibition in all JAK2V617F PV and ET patients at 0.01 µM concentration while SAHA displayed a similar inhibitory activity only when used at 0.25 µM. When the EEC assay was performed using cells from JAK2V617F mutated PV or ET patients in the presence of exogenous cytokines, the addition of ITF2357 allowed the growth of JAK2V617F unmutated colonies which could account up to 60% of total colonies. Discussion. ITF2357, at low in vitro concentration, show a potent inhibitory activity on the autonomous proliferation of hematopoietic stem cells of PV and TE carrying JAK2V617F mutation. This holds promise for a possible clinical use of this molecule in chronic myeloproliferative disorders.

**PO-181**

**SAHA INHIBITS PROLIFERATION AND INDUCES APOPTOSIS IN A MEGAKARYOBLASTIC CELL LINE AND SYNERGISTICALLY INTERCATCS WITH THE PORTEASOME INHIBITOR BORTEZOMIB.**

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Histone deacetylase inhibitors, favouring histone acetylation and chromatin relaxation, thereby allowing the transcription of genes involved in the control of cell proliferation, differentiation and apoptosis, represent a promising group of anticancer agents. Suberoyanilide hydroxamic acid (SAHA) seems to exert an antitumor effect in a synergistic manner with topoisoosmerase inhibitors. TRAIL and other several anticancer agents. Bortezomib is a dipeptide boronic acid proteasome inhibitor with proved antitumor activity in multiple myeloma and able to induce apoptosis in different human cancer cell lines. In the present study we investigated the anti-proliferative and pro-apoptotic effects of SAHA in combination with bortezomib on a megakaryoblast cell line (Mo7-e). Microarray tests were also performed in order to better understand the molecular mechanisms. A dose-dependent inhibition of cell proliferation was observed and a synergistic interaction by both bortezomib and SAHA was detected. The effect of analysis of interactions between SAHA and bortezomib administered over a range of concentrations (0.5-5 uM and 5-20 nM, respectively) showed a combination index value <1, corresponding to a synergistic interaction. Analogously, 45% and 47% of apoptotic cells were observed after exposure to IC50 concentrations of SAHA and bortezomib. The co-exposure significantly increased the percentage of apoptotic cells up to 60%, while microarray results showed that SAHA and bortezomib act on different chemokine receptors (CCR3, CCR7) and several pro-apoptotic factors, such as ATF3 and TRAIL receptor. Moreover, it down-regulate NF-κB, BCL2, P53, JAK3, and WT1 gene expression. On the other hand, bortezomib up-regulates Ik-B, ATF3, and down-regulates PDGF and PDGFR, GST, TOPOISOMERASE IIa, BCL2, and WT1 gene expression. The association between the two agents was able to up-regulate Bax expression and down-regulate WNT5B, n-RAS, THROMBOXANE A SYNTHASE, THROMBOPOIETIN, THROMBOMODULIN, and WT1. About this last gene, the inhibition of the mRNA expression resulted synergistic when the cells were incubated with two drugs concomitantly (data confirmed by quantitative real-time PCR assays). Finally, we observed a cell cycle block in the G2 phase exerted by bortezomib and in the G1 phase when cells were incubated with SAHA. Microarray assays showed a down-regulation of CYCLINS D1, D2 and D3 by bortezomib and of cyclins A1, D2 and D3 by SAHA. Studies about protein expression will be necessary for completing the study; nevertheless, the above reported results appear particularly relevant for the possible efficacy of SAHA and bortezomib (already adopted for treatment of other malignancies) also in the megakaryocyte-driven chronic myeloproliferative disorders.
resulted in the expression of a membrane-anchored hyperactive Ras protein able to activate the MAPK cascade, suggesting oncogenicity of the novel mutation. Discussion. In our series of patients RAS and JAK2 mutations were quite frequent in MP-CMML and always mutually exclusive. Because MP-CMML may evolve from MD-CMML, these findings support the hypothesis that molecular abnormalities could be acquired with disease progression. Since both JAK2 and RAS proteins are involved in the GM-CSF signalling pathway, the higher levels of intracytoplasmic cytokine and the increased density of its receptor in MP-CMML support the hypothesis that this pathway is crucial in malignant cell proliferation of CMML pts.

PO-183

INFLUENCE OF THE JAK2 V617F HOMOZYGOUS OR HETEROZYGOUS MUTATION ON THE THROMBOTIC RISK AMONG PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA


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Introduction. It is uncertain whether the JAK2 V617F mutation is associated with an increased risk of thrombosis in patients with Philadelphia-negative hematopoietic malignancies. Recent studies have shown that the homozygous status has been reported to increase the risk in patients with essential thrombocythaemia (ET). It is unknown whether inherited thrombophilia is an additive risk factor in the patients with the JAK2 mutation. The present study is aimed to investigate the thrombotic risk associated with the JAK2 mutation and thrombophilia in ET patients.

Patients and Methods. We studied 132 patients with essential thrombocythaemia who were followed in the Haematology Unit, Clinical Hospital of Bari between January 1998 and December 2005. The JAK2 V617F mutation was detected in 116 patients (88%). The diagnosis of ET was established according to the WHO criteria.

Conclusions. In this study, we found the presence of palpable hepatosplenomegaly in 7, peripheral arterial thrombosis in 18 cases (splanchnic veins in 9, deep veins of the legs in 7, cerebral veins in 1, retinal vein in 1). All patients were investigated for the presence of the JAK2 V617F mutation by PCR; the mutational allele burden higher or lower than 80%. Laboratory investigation for inherited thrombophilia (deficiency of antithrombin, proteins C and S, factor V Leiden [FVL], prothrombin G20210A [PT-A]) was carried out in all patients. We analyzed the relative risk (RR) for thrombosis according to the presence of the JAK2 mutation and/or thrombophilia. The risk for thrombosis was further estimated according to Homo or Hetero for the JAK2 mutation. To increase the statistical power of such latter analyses, we investigated additional 81 patients with polycythemia vera (PV) and the JAK2 mutation, 39 of them having had a thrombotic event. Results. The JAK2 mutation was detected in 83 ET patients (62.8%), with Homo in 4 cases (4.8%). Seven patients carried thrombophilia (4 FVL and 3 PT-A). The RR for thrombosis was 2.1 (95% CI 1.1-3.8) in JAK2 mutated patients with homozygous status compared to WT patients; in Homo and Hetero the RR was 3.7 (95% CI 1.7-8.1) and 2.0 (95% CI 1.7-3.7) in comparison with WT patients. The RR of Homo in comparison with Hetero bordered statistical significance (1.8, 95% CI 1.0-3.5). Such RR values were substantially unchanged after adjustment for the presence of thrombophilia. In patients with mutation and with thrombophilia the RR was 4.4 (95% CI 2.2-8.6) in comparison with WT patients without thrombophilia. Among the 81 PV patients with JAK2 mutation, 14 carried Homo and thrombophilia (8 FVL and 2 PT-A). Overall, in combined PV and ET patients the RR associated with Homo was 1.7 (95% CI 1.2-2.4) in comparison with Hetero and 1.6 (95% CI 1.1-2.5) after adjustment for thrombophilia. Conclusions. In ET patients the thrombotic risk is higher in the presence of the JAK2 mutation. The magnitude of the increase in risk is dependent on the mutant allele burden, being higher in homozygotes. The concomitant presence of inherited thrombophilia does not seem to produce substantial variations, yet further studies on larger patient cohorts are needed to confirm this finding.

PO-184

ATYPICAL CHRONIC MYELOID LEUKEMIA (aCML): DESCRIPTION OF 55 CASES AND IDENTIFICATION OF PROGNOSTIC FEATURES


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According to WHO classification the diagnosis of aCML is made when a marked granulocytosis with multilineage dysplasia is found in the absence of the Philadelphia chromosome and BCR/ABL fusion gene. Among so far reported cases of aCML, a wide spectrum of presenting features was observed, with some patients meeting all the WHO criteria, and others showing some discrepancies. We analyzed our series of 55 patients diagnosed as having aCML, with the aim of identifying clinical factors of possible prognostic value on survival and acute transformation. All our patients included in present study fulfilled the WHO diagnostic criteria for the diagnosis of aCML: persistent leukocytosis, lack of BCR/ABL fusion gene, evidence of multilineage dysplasia, monocyto- tty <1×10^9/L, basophilia <2%, immature circulating precursors (ICP) >10%, BM blasts <20%. Median patient age was 62 years, with 15 patients under 65 years (p=0.001), male sex (57% vs 43%). Median leukocyte value (25.7×10^9/L, range from 24 to 76×10^9/L) was similar to those of other patient series but was lower if compared to that of about 100×10^9/L reported by the FAB group. The most common karyotypic changes observed in aCML include abnormalities of chromosomes 8, 13, 14, 17, 19 and 21; in our series, the most consistent changes were chromosome 20q deletion (7 patients) and trisomy 8 (4 patients). At the time of this writing, 51 patients have died and 24 are still alive, overall median survival being 25 months. Acute transforma- tion occurred in 22 patients (40%). In multivariate analysis a shorter survival was associated with older age (>65 years, p=0.04), female sex (p=0.0001), leukocytes >50×10^9/L (p=0.001), presence of immature circu- lating precursors (ICP, p=0.05); as predictive factors for risk of evolution, we found the presence of palpable hepatosplenomegaly (p=0.03), monocyto- tty (3<5<8% with monocytes <1×10^9/L, p=0.05), BM blasts >5% (p=0.007), marked dyserythropoiesis (p=0.004), and trans- fusional requirement (p=0.01). The diagnosis of aCML still remains restricted to Ph, BCR/ABL negativity and essentially to PB and BM cyto- logical features. In the absence of new molecular markers, the identifi- cation of factors capable of delineating distinct clinical outcomes, might be of practical usefulness. Thus, present data should be prospectively considered for validation in larger series of aCML patients.

PO-185

FLOW CYTOMETRIC DETECTION OF CIRCULATING ENDOTHELIAL CELLS IN CHRONIC MYELOPROLIFERATIVE DISORDERS

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A growing body of evidence supporting an important role of angiogenesis and neovascularization in the pathophisiology of a wide array of diseases has led to an increasing interest in measuring circulating endothelial cells (CEC). Most early studies of CECs relied solely on morphological identification of cell type. Flow cytometry is clearly a suitable method for the detection and quantitation of rare events such as CECs but despite many recent studies, questions remain regarding the precise cell surface markers that unequivocally identify these cells. The aim of this study was to use a multiparameter flow cytometry and sequential gating strategy to measure CECs levels in a series of patients affected by chronic myeloproliferative disorders (CMD) and healthy controls. Informed consent was given by all subjects. To avoid contamination by endothelial cells from the punctured vessel wall, the first 2 ml of blood drawn were discarded. Forty-six samples were collected [34 patients affected by CMD (27 myelofibrosis with myeloid metaplasia, 4 essential thrombocythemia, 3 polycythemia vera), and 12 healthy subjects] and stained with CD31 FITC, CD133 PE, CD34 ECD and CD45 PerCP. CECs were defined as CD34 positive events, lacking CD45 and with expression of CD31. The live-no-wash procedure was used and for each sample a minimum of 200,000 CD34 positive events were acquired by the FC-500 flow cytometer and analyzed by CXP software; absolute
cell numbers were calculated by reference fluorescence beads. In addition, numbers of circulating hematopoietic progenitor cells (HPCs) were measured in all samples according to ISHAGE guidelines. Five samples were also stained with CD105 PE or CD41/CD61 PE instead of CD133 PE. Healthy subjects had levels of CECs ranging from 0 to 303/mL with a median value of 73 while HPCs ranged from 1 to 5/microL; CEC counts in patients with CMD samples ranged from 75 to 11,636/mL with a median value of 682/mL while HPC values ranged from 2 to 631 μL with a median value of 24 μL; differences in HPC and CEC levels between essential thrombocythemia or polycythemia vera patients and controls were not significant while patients with myelofibrosis with myeloid metaplasia showed significantly higher HPC and CEC counts than healthy subjects (p=0.001 and 0.05, respectively). The unexpectedly high values of CECs in healthy subjects and the expression of CD41 and CD61 in most of the CD31+/CD34+/CD45– events and of CD105 only in a small proportion of them revealed that most of the cells identified as CECs were actually platelets, as already suggested by Strijbos MH et al. (Cytometry B Clin Cytom, 2007). We restudied flow cytometric methods of detecting CECs and suggest that, although redundant endothelial markers could increase the accuracy of CECs detection, a single platform whole blood assay where CECs are identified as events CD45+/CD34+/CD31+/CD105– could be feasible for correctly enumerating CECs. Further studies on endothelial colony assays performed on CD34– selected cells could be useful to confirm that CD34 cells expressing CD31 and CD105 and lacking CD45 are endothelial cells and not large platelets.

**PO-186**

**CLINICAL AND LABORATORY DIFFERENCES AT DIAGNOSIS AND DURING FOLLOW-UP BETWEEN JAK2(V617F) POSITIVE AND NEGATIVE PATIENTS WITH UNCLASSIFIABLE CHRONIC MYELOPROLIFERATIVE DISORDERS: A SINGLE CENTRE EXPERIENCE**

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Introduction. According to WHO classification, unclassifiable chronic myeloproliferative disorders (uMPD) are a subgroup of diseases showing a multi lineage proliferation without typical characteristics of any other Philadelphia negative MPD. Recently, an activating mutation of the JAK2 tyrosine Kinase (V617F) has been well described in about 90% of patients (pts) with polycythemia vera and in less than half of pts with essential thrombocythemia and idiopathic myelofibrosis. The role of JAK2(V617F) mutation in uMPD remains still unclear. Methods. 21 uMPD pts referred in our hematology unit from 1994 to 2006 were screened for JAK2 mutational status, using allele-specific PCR (cfr Baxter, et al. Lancet, 2005), in order to compare clinical and laboratory features at diagnosis and during follow-up between JAK2 positive and negative pts. Comparisons between categorical and continuous variables were performed by chi-squared statistics and by Wilcoxon rank-test, respectively. Results. We identified 13 (62%) positive and 8 (38%) negative JAK2 pts. There was no significant difference in spleen enlargement (median 1 cm below costal margin), sex (M:F ratio 0.8 vs 6.2), white cell count (WBC 10.4 vs 16.8×10^9/L; p=0.09) and LDH level (454 vs 587 U/L; p=0.54) at diagnosis between JAK2 positive and negative pts, respectively. In contrast, pts positive for JAK2 mutation had at diagnosis significantly older age (68 vs 49 years; p=0.054), higher hemoglobin level (Hb 16.9 vs 12.5 g/dL; p=0.004) and PLT count (656 vs 20010/μL; p=0.014) than negative pts. Thrombotic complications were more common in pts with JAK2(V617F) mutation (4/13 pts, 30.7 vs 0 pts). A total of 4 leukemic transformation (19%) were detected, 3 in JAK2 negative pts (37.5%) and one in JAK2 positive pts. At present, all JAK2 negative pts were alive, while 2/13 pts (15.4%) JAK2 positive died. Discussion. Our experience suggests the existence of different features at diagnosis and during course of disease, according to JAK2 mutational status, in uMPD pts. JAK2 mutation is present in 62% of our pts and defines a subgroup with older age and a polycythemic phenotype at diagnosis and thrombotic tendency in the follow-up of disease. Moreover pts positive for JAK2 mutation seem to have a worse outcome (15.4% of deaths vs 0%), even if the incidence of leukemic transformation seems to be higher in JAK2 negative group. Additional analysis and larger studies needs to confirm our results.

**PO-187**

**LEUKOCYTE ALKALINE PHOSPHATASE EXPRESSION PREDICTS THE JAK2(V617F) MUTATION STATUS IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHESMA**

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Introduction. A single somatic mutation in the Janus Kinase 2 gene (JAK2(V617F)) has been identified as the key pathogenetic event in Polycythemia Vera (PV) and Essential Thrombocythemia (ET). The Leukocyte Alkaline Phosphatase (LAP) and the Polycythemia Rubra Vera 1 (PRV-1) genes are remarkably up-regulated in most patients with PV and some with ET. Aim of this study was to predict the presence of the JAK2(V617F) mutation by flow cytometry analysis of LAP expression. Methods. Patients with PV (n=146), ET (n=529) and Idiopathic Erythrocyesis (IE, n=56) were selected among those regularly followed up in our clinic. Healthy volunteers were selected from those referred to our institutional blood bank for blood donation (n=40). The JAK2(V617F) mutation screening was performed by allele specific Polymerase Chain Reaction (PCR). The surface expression of LAP was quantified as arbitrary units of Mean Fluorescence Intensity (MFI) by flow cytometry while PRV-1 gene expression was performed by Real Time Quantitative PCR (RQ-PCR) using RNA from purified granulocytes. The discriminant ability of LAP and PRV-1 expression in identifying the JAK2(V617F) mutation was performed by a Receiving Operating Characteristic (ROC) analysis. Results. The mutational analysis for JAK2(V617F) showed that 90% of PV and 53% of ET patients were mutated, while none of the IE patients proved positive. The LAP expression was detected on granulocytes isolated from IE and ET patients lacking the JAK2(V617F) mutation was not different from that of normal donors and a progressive and highly significant increase of the LAP MFI was registered in ET and PV patients carrying a heterozygous or homozygous JAK2(V617F) mutation. A ROC curve analysis allowed to identify a LAP MFI value of 90 as the optimal cut-off to discriminate, with a good score of sensitivity and specificity, patients carrying a JAK2 wild type gene as compared to those with a JAK2(V617F) mutation. The expression of a homozygous JAK2(V617F) mutation strongly correlates with the highest values of LAP expression. The simultaneous RQ-PCR evaluation of PRV-1 gene expression in peripheral blood granulocytes provided evidence of a remarkable linear correlation of results obtained by these assays. The ROC curve analysis by plotting results of both the LAP and the PRV-1 tests gave superimposable results. Discussion. Flow cytometry of LAP on peripheral blood granulocytes is an easy and reproducible assay to predict the JAK2(V617F) mutation.

**PO-188**

**INCIDENCE OF SYSTEMIC MASTOCYTOSIS IN PTS WITH HYMENOPTERA VENOM ALLERGY AND RAISED SERUM TRYPTASE LEVELS: ROLE OF FLOW-CYTOMETRY**


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Patients with systemic mastocytosis (SM) may suffer from severe anaphylactic reactions after wasp or bee stings. We evaluated the incidence of SM in patients with allergic reactions after hymenoptera bites and high serum tryptase basal levels. We analyzed the serum tryptase basal levels in 276 patients with previous anaphylactic reactions due to hymenoptera bites. Bone marrow (BM) aspirate (stained according to May-Grunwald-Giemsa method) and biopsy (with anti-tryptase monoclonal antibody staining to identify atypical MC infiltration) were carried out in patients with elevated serum tryptase levels (>11.4 ng/mL). In addition, multiparametric flow cytometric analysis was applied to BM samples to identify MCs (CD45+/CD117+/CD34) and to evaluate abnormal expression of CD25 and CD2 on MCs. Finally, we assessed the presence of D816V KIT mutation in BM mononuclear cells by restriction fragment length polymorphism analysis. Tryptase levels were increased (median 122.6 ng/mL; range 1.7-10,276 ng/mL) in 22/39 patients (56.4%). Twenty-three of these patients were studied with BM analysis (Table 1): 20 had previous severe anaphylactic reactions (type III: 2 cases, type IV:...
18 cases). BM immunohistochemistry showed typical infiltrates of tryptase+ spindle-shaped cells in 10/25 patients (45%). The KIT mutation D816V was found in 13/21 (62%) cases. BM smear showed atypical MCs in 13/21 (62%) patients. In 18/21 (86%) cases, BM CD117+ MCs, evaluated by flow-cytometry, expressed CD2 and/or CD25. By contrast, MCs from normal BM (n=2), other haematological malignancies (n=4), and cutaneous mastocytosis (n=4) did not express CD2 and/or CD25. Cytogenetic analysis was normal in all cases. In summary, the final diagnosis was indolent SM (ISM) according to WHO criteria in 16 patients (70%). In 3 patient only two minor criteria were found, and 2 patient showed only the aberrant expression CD25/CD2 on MC; these patients were considered affected by Monoclonal Mast Cell activation syndrome (MMCAS), as proposed by Valent et al. (2007). Only two patients (9%) didn’t show signs of abnormal MC infiltration. Our results show strong association among anaphylaxis due to hymenoptera bites, abnormal basal serum tryptase levels and SM. In

<table>
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Ciancia R,1 Martinelli V,1 De Angelis B,2 Picardi M,1 Montante B,1 Ciancia G,1 Fabbricini R,1 Gherghi M,1 Pane F,1 Rotoli B1

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Chronic myeloproliferative diseases (CMDs) are heterogeneous clonal hematopoietic stem cells disorders characterized by the expansion of a single neoplastic stem cell. The molecular basis of several myeloproliferative diseases is unknown. On the basis of the model of CML, we may surmise that a constitutive activation of a tyrosine kinase might be involved even in other CMDs. Recent data have confirmed that a somatic mutation at position 617 (V617F) of the Janus kinase 2 (JAK2) gene may be found in the majority of PV and in a sizeable proportion of ET and IMF. These findings can lead to new perspective for diagnosis and classification of CMD patients, and, in future, even for their treatment. We screened for V617F bone marrow samples of 83 ET, 34 PV and 4 MF patients, banked at CEINGE- Biotecnologie avanzate in Naples, collected by the Hematology Division. The samples were sequenced to identify the V617F JAK2 mutation derived from the G-to-T mutation at position 1849; a sample was considered positive if the mutation was present in at least 20% of cells. The V617F JAK2 mutation was detected in 30 of the 34 PV patients (88%), in 47 of the 83 ET patients (57%) and in 2 of the 4 MF patients. As expected, in most ET cases the signal of the G-to-T mutation was weak: clonal hematopoiesis is rarely characterized by a large expansion in ET patients. Many authors have shown that patients with V617F positive CMD have a longer median disease duration and were more likely to develop complication such as secondary myelofibrosis, bleeding or thrombosis than those without the mutation.

Moving from these data we looked for correlation among clinical disease features (i.e. spleen volume, LDH and marrow fibrosis grading) and genotype (wild-type (wt) versus mutant (m)) in PV and ET patients. We found that the median level of LDH was significantly higher in V617F positive cases. We did not found a statistically significant difference for spleen volume in the two groups of patients, although the median value of spleen volume in mutant patients was higher than in the wild-type group. We found that fibrosis grading distribution was different among the two groups: in the mutant group 94% of ET patients and 87% of PV patients had fibrosis grade 0-1 and 6% of ET patients and 12.5% of PV patients grade 2; in the wild type group fibrosis was always 0-1, never grade 2. Wider studies are needed to confirm these correlations. In our data JAK2 mutation also affects response to treatment. Among ET patients, those with the JAK2 mutation are less sensitive to hydroxyurea.

The relationship between the V617F mutation and the risk of progression in patients affected by CMD could not to be determined yet in our small series with a short follow up time. The V617F mutation of JAK2 is a marker for disease diagnosis and a tool for monitoring the disease. Different approaches are required to identify markers in the group of patients without JAK2 mutation.

**PO-192**

CONGENITAL HYPOFIBRINOGENEMIA ASSOCIATED WITH HETEROZYGOUS FIBRINOGEN BBETA AND GAMMA CHAIN MUTATIONS

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Fibrinogen is a complex glycoprotein involved in the final step of the coagulation cascade as the thrombin substrate for fibrin generation. Fibrinogen is synthesized in hepatocytes as a hexamer composed of two sets of three polypeptide chains (Aalpha, Bbeta and gamma). Each chain is encoded by a distinct gene (FGA, FGB and FGG). Three different mutations in heterozygosity were found. The first patient (fibrinogen 70 mg/dL) presents a novel non-sense mutation in exon 8 of FGB, a transition nt. 7893 CAG>TAG predicting a Gln263X3 in heterozygosity. This mutation causes a truncated fibrinogen Bbeta chain, which may not be assembled in the fibrinogen molecule. The second patient (fibrinogen 70 mg/dL) presents a novel heterogeneous transition nt. 7611 GAT>GGT in exon 8 of FGG, leading to the missense mutation Arg326Stop. The third patient (fibrinogen 70 mg/dL) presents a novel non-sense mutation in heterozygosity. This mutation causes a truncated fibrinogen Bbeta chain, which may not be assembled in the fibrinogen molecule. The third patient (fibrinogen 70 mg/dL) presents a novel heterogeneous transition nt. 7611 GAT>GGT in exon 8 of FGG, leading to the missense mutation Arg326Stop. The third patient (fibrinogen 70 mg/dL) presents a novel heterogeneous transition nt. 7611 GAT>GGT in exon 8 of FGG, leading to the missense mutation Arg326Stop.

**Table 1. Phenotype and genotype in five patients with severe FXIII deficiency.**

<table>
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<tr>
<th>PT</th>
<th>SEX</th>
<th>AGE</th>
<th>SYMPTOMS</th>
<th>FIXD ACTIVITY</th>
<th>DNA MUTATIONS</th>
<th>DOMAIN</th>
</tr>
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<td>A</td>
<td>M</td>
<td>16</td>
<td>Recr Hematomas</td>
<td>&lt;3%</td>
<td>2103-2104 Del CT homo Lys667T/C5</td>
<td>Barel 2</td>
</tr>
<tr>
<td>B</td>
<td>M</td>
<td>16</td>
<td>Recr hematomas, intracranial bleeding</td>
<td>&lt;5%</td>
<td>nt.661 CDT&gt;CTT</td>
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<tr>
<td>C</td>
<td>F</td>
<td>44</td>
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<td>&lt;3%</td>
<td>nt.2237 AGD&gt;AGC Ser706Ala homo</td>
<td>Barel 2</td>
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<td>D</td>
<td>M</td>
<td>4</td>
<td>Recr hematomas, umbilical cord and post surgical bleeding</td>
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<td>Core</td>
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<td>E</td>
<td>F</td>
<td>31</td>
<td>Umbilical cord and post surgical bleeding</td>
<td>&lt;4%</td>
<td>nt.1088 GGA&gt;TGA Arg326Stop homo</td>
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PO-193
RITUXIMAB INDUCED THROMBOCYTOPENIA IN MARGINAL LYMPHOMA AND HAIRY CELL LEUKEMIA: A RARE, BUT POSSIBLE COMPLICATION. REPORT OF FOUR CASES
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Introduction. Rituximab is an anti-CD20 monoclonal antibody used CD20+ NHL therapy, generally well tolerated. Common side effects include anaphylactoid infusion reactions. Although episodes of neutropenia have been reported after rituximab infusion, isolated acute thrombocytopenia is extremely rare. Methods. We describe transient acute thrombocytopenia after rituximab infusion in 4 patients: 2 with Haicy cell leukemia and 2 with splenic marginal zone lymphoma. M/F was 2/1, median age was 78 years (range 67-78). All patients showed no severe splenomegaly. They were treated with Rituximab (375 mg/m²) on day 0 of therapy. Three of these showed fever, cardiopalmus, chills and other symptoms related to cytokine-release syndrome during or immediately after monoclonal antibody administration. Results. We observed severe acute thrombocytopenia development on the first day following rituximab administration. Patient platelet counts at hospital admission were 88,000/mmc, 65,000/mmc (2 patients), 84,000/mmc, 53,000/mmc respectively. All patients recovered normal platelet count in a median time of 4 (4-6) days after the administration of the monoclonal antibody. None received platelet transfusion, showed other CBC alterations or had major or minor hemorrhagic manifestation. Coagulation parameters and C3, C4, IC dosage, analyzed immediately after monoclonal antibody administration. None of the patients showed an alteration of the von Willebrand factor. Discussion. Previous reports suggest that Rituximab related thrombocytopenia is due to CD20 antigen adsorption, after cytolsis, on platelets surface. An alternative hypothesis considers a complement activation by raise of soluble CD20 in the circulation after cytolsis, but in our case-series normal values of C3, C4 and IC dosage seem to contradict this. The presence in scientific literature of other reports referring Rituximab related thrombocytopenia in hairy cell leukemia, mantle cell lymphoma and chronic prolymphocytic leukemia, but not in other most frequent B-cell lymphoma, suggests that Rituximab related thrombocytopenia, although is a rare complication, it’s expected especially in less frequent lymphomas.

PO-194
MEGAKARYOCYTE APOPTOSIS AND NOXA ACCUMULATION AS A CAUSE OF B RITUXIMAB-INDUCED THROMBOCYTOPENIA
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The proteasome inhibitor Bortezomib exhibits antitumor activity against tumors of different histology, including solid and hematologic malignancies. One of the most common toxicities after Bortezomib therapy is severe thrombocytopenia (grade 3-4) which occurs in approximately 30% of patients and may require treatment suspension, dose reduction or platelet transfusions. Recently, we have shown that thrombocytopenia induced by conventional chemotherapeutic agents occurs through a selective cytotoxic effect of antineoplastic drugs on immature megakaryocyte progenitors (Zeuner A et al., Cancer Research 2007). Here we have investigated the effect of Bortezomib on primary megakaryocytes by using a culture system that, starting from CD34, recapitulates in vitro human megakaryopoiesis. We found that megakaryocytes at early stages of differentiation were primarily sensitive to Bortezomib which displayed a dose-dependent apoptotic effect on mononucleated megakaryocytic progenitors. In primary megakaryocytes Bortezomib treatment was associated with a strong induction of expression of the protein Noxa, a proapoptotic Bcl-2 family member that promotes mitochondrial outer membrane permeabilization, thus indicating a possible mechanism of Bortezomib-induced apoptosis. Importantly, the hematopoietic cytokine Stem Cell Factor (SCF), which is a potent anti-apoptic factor for immature hemopoietic cells, was able to protect immature megakaryocytes from Bortezomib-induced apoptosis. These results provide an explanation for the occurrence of thrombocytopenia in Bortezomib-treated cancer patients and may support the development of therapeutic strategies to prevent platelet depletion in cancer patients.

PO-195
HIGH PREVALENCE OF INHERITED SEVERE FXI DEFICIENCY IN ABRUZZO REGION: MOLECULAR GENOTYPING
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Background. Severe Factor XI deficiency is a rare (1:100,000) autosomal inherited bleeding disorder characterized by a heterogeneous bleeding tendency, often independent from circulating FXI levels. More than 100 mutations in FXI gene have been found so far (http://www.factordx.com). Two prevalent mutations (Glu117X and Phe283Leu), account for the majority of abnormal alleles in Jews, while large genetic heterogeneity is found in non-Jewish patients. We report here the genetic characterization of six unrelated patients with severe FXI deficiency (FXI:C ≤2%) from the Abruzzo Region. Methods. Factor XI activity was measured by an aPTT-based assay with severe factor XI-deficient plasma as substrate. The whole coding sequence, intron-exon boundaries and the promoter region of FXI gene were sequenced in six patients with severe FXI deficiency. None of Jewish ancestry. Bleeding symptoms were evaluated by using a standardized questionnaire, recently designed for assessment of bleeding score in von Willebrand disease (normal range in 195 controls <5). Results. Candidate mutations were identified in all the patients. The Jewish type II mutation (Glu117Stop) was found in homozygosity in a patient only. All the remaining were compound heterozygotes (novel mutations underlined): Phe283Leu with Glu116X, Thr128Met with Glu117X, Thr128Met with Cys122-Cys128 disulfide pairing in Apple 2 domain responsible for substrate binding in FXIa. Cys506X disrupts Cys503-Cys509 pairing in Apple 4 domain. The introduction of the new cysteine residue 578 occurs near the Cys351-Cys358 pairing in the catalytic region. The bleeding scores obtained from bleeding symptoms were substantially similar to most of normal controls, again confirming that the severity and account for the bleeding symptoms is completely different from what is observed in patients with other severe deficiencies of clotting factors. Conclusions. The unexpected high prevalence of severe FXI deficiency in Abruzzo Region (about 5 cases /1,000,000) is associated with a wide allelic heterogeneity in FXI gene.

PO-196
CELLULAR AND SOLUBLE MARKERS OF ENDOThelial DYSFUNCTION IN PATIENTS WITH THROMBOTIC THROMBOcytopenic PURPURA IN REMISSION
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Background. Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disorder characterized by microangiopathic hemolytic anemia and thrombocytopenia as a result of microvascular platelet clumping. Severe deficiency of the von Willebrand factor (vWF) – cleaving protease, ADAMTS-13, prevents normal processing of unusually large vWF multimers released from endothelial cells and it is assumed that their persistence is responsible for the formation of platelet thrombi in the microvasculature. It has also been suggested that endothelial apoptosis is a primary lesion in the pathogenesis of TTP. Aims. Endothelial Progenitor Cells (EPCs) and Circulating Endothelial Cells (CECs) might be used as a surrogate marker for the study of vascular alterations. CECs are identified as CD34+/CD146+/CD105+/CD11b–, while EPCs displays late colony formation/ outgrowth on fibronectin coat. Co-expression of KDR and CD34 characterized EPCs. Methods. We investigated 14 TTP patients with complete remission of disease, evaluating plasma ADAMTS-13 activity, EPCs plasmatic level and colony formation and
Cyclic thrombocytopenia (CTP) is a rare disorder characterized by periodic episodes of thrombocytopenia, often symptomatic, followed by spontaneous recovery to normal platelet counts. CTP is more frequent among women, mainly pre-menopausal, and is often associated with menstrual periods. It may be associated with other haematological diseases, but a majority of cases are idiopathic. In some patients, cyclic platelet fluctuations can parallel periods of amegakaryocytosis. The disorder is typically chronic and is rarely associated with life-threatening bleeding episodes. The pathogenesis of cyclic thrombocytopenia is under debate. Immunological and hormonal factors are hypothesized. However, complete remissions after immunosuppressive or hormonal treatment are not very frequent. Rare congenital forms are also described. We recently diagnosed CTP to a 65yr-old woman, affected by Pure Red Cell Aplasia (diagnosed in 1999) in transfusional support, and Felty syndrome (diagnosed at the age of fourteen years). The patient, who had been splenectomised in 2002 and had shown to be insensitive to several immunosuppressive attempts (CSA, mofetil micofenolate, infliximab), was found to have 50.000 platelets/mm^3 at control visits in July 2006. Counts spontaneously recovered after 8 days, but low levels (nadir 23.000/mm^3) were again found in September and December 2006, January and April 2007, always followed by spontaneous rapid normalization. Pseudothrombocytopenia by EDTA was excluded; megakaryocytes were not detected, but the significance of narrow analysis was reduced by the absence of specific platelet reduction was in some cases mildly symptomatic (bruising). Anti-platelet antibody were absent. In our patient, the suggestion of autoimmune pathogenesis of CTP is particularly strong. However, the phenomenon appears self-limiting and does not require treatment to date. The question whether in our case CTP represent a further step to wider damage of the hemopoietic tissue is still open.

PO-197

PSYMPHOMATIC MARKERS AND THROMBO-HAEMORRAGIC COMPLICATIONS IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA (APL)

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Introduction. Differentiating therapy with all-trans-retinoic acid (ATRA) induces the remission of this disease and resolves the life-threatening coagulopathy typical of APL. Aim of the study was to prospectively follow for 8 weeks newly diagnosed APL patients to evaluate: 1. The rate of hemorrhage and thrombosis; 2. The plasma levels of thrombotic markers before and after starting induction therapy; 3. The predictive value of these markers. Methods. Forty-six consecutive patients with APL (F/M = 21/25; age range = 8-84 years) were enrolled at our Centre from January 2000 to April 2007. All patients received induction therapy with Idarubicin + ATRA. Plasma samples were obtained from 21/46 APL patients, at baseline (T0), and at day 7 (T7), 15 (T15), and 25 (T25) after starting ATRA. Thrombotic markers, measured by ELISA, included: D-dimer, thrombin-antithrombin complex (TAT), tissue factor pathway inhibitor (TFPI), and activated factor VII-antithrombin complex (FVIIa-AT). At disease onset, 8/46 patients had severe haemorrhages (17.3%), including 3 (6.5%) fatal intracranial bleeding and 5 non-fatal major bleedings (10.6%); 3/46 patients (6.5%) had thrombosis [1 fatal Budd-Chiari syndrome (2.1%), and 2 non-fatal events (4.3%)]. During induction therapy, 3 patients (6.5%) developed thrombosis at day 9, 15 and 46, respectively. The laboratory study in the sub-group of 21 patients showed that, at T0, the levels of plasma markers were all significantly (>0.05) lower than in healthy subjects (n=25). During ATRA therapy D-dimer and TAT were significantly (>0.05) reduced at T7 compared to T0, whereas TFPI levels significantly decreased at T15 (vs T0, >0.05). FVIIa-AT showed a significant reduction later, at T25 (vs T0, >0.05). Three of the 21 patients had thrombosis, after 1, 9, and 15 days of ATRA, respectively, none had major haemorrhages. The treatment TFPI levels >74.9 showed a significant predicting value for thrombosis by the Fisher’s exact test (RR=4.33; CI=33.30-92.30, >0.05). Discussion. These data confirm a significant rate of early deaths in APL due to the coagulopathy. The analysis of coagulation markers shows for the first time persistent elevated levels of TFPI and FVIIa/AT, possibly due to persistent APL cell TF expression. In addition, TFPI levels at diagnosis demonstrate a promising role as a marker of increased thrombotic risk. This result deserve to be tested by large prospective clinical studies.

PO-198

CYCLIC THROMBOCYTOPENIA DURING PURE RED CELL APLASIA

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Cyclic thrombocytopenia (CTP) is a rare disorder characterized by periodic episodes of thrombocytopenia, often symptomatic, followed by spontaneous recovery to normal platelet counts. CTP is more frequent among women, mainly pre-menopausal, and is often associated with menstrual periods. It may be associated with other haematological diseases, but a majority of cases are idiopathic. In some patients, cyclic platelet fluctuations can parallel periods of amegakaryocytosis. The disorder is typically chronic and is rarely associated with life-threatening bleeding episodes. The pathogenesis of cyclic thrombocytopenia is under debate. Immunological and hormonal factors are hypothesized. However, complete remissions after immunosuppressive or hormonal treatment are not very frequent. Rare congenital forms are also described. We recently diagnosed CTP to a 65yr-old woman, affected by Pure Red Cell Aplasia (diagnosed in 1999) in transfusional support, and Felty syndrome (diagnosed at the age of fourteen years). The patient, who had been splenectomised in 2002 and had shown to be insensitive to several immunosuppressive attempts (CSA, mofetil micofenolate, infliximab), was found to have 50.000 platelets/mm^3 at control visits in July 2006. Counts spontaneously recovered after 8 days, but low levels (nadir 23.000/mm^3) were again found in September and December 2006, January and April 2007, always followed by spontaneous rapid normalization. Pseudothrombocytopenia by EDTA was excluded; megakaryocytes were not detected, but the significance of narrow analysis was reduced by the absence of specific platelet reduction was in some cases mildly symptomatic (bruising). Anti-platelet antibody were absent. In our patient, the suggestion of autoimmune pathogenesis of CTP is particularly strong. However, the phenomenon appears self-limiting and does not require treatment to date. The question whether in our case CTP represent a further step to wider damage of the hemopoietic tissue is still open.

PO-199

RETROSPECTIVE STUDY OF 207 PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA: THE ROLE OF SPLENECTOMY

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We retrospectedly analyzed the data on 207 pts with immune thrombocytopenic purpura (ITP). The median platelet count at diagnosis was 38 (range 0-130). The median age at diagnosis was 47.9 (range 4-87). Twenty-three pts were positive for HBV and 13 for HCV. 33 pts were positive for antiplatelet-antibody, 14 for LAC, 31 for ANA, 6 for ACA, 4 for Amphi, 4 for ENA, 4 for SCAPA, 6 for antiphospholipid autoantibody, 3 for thyreoglobuline autoantibody. 5 pts were positive for Helicobacter pylori: antibiotic therapy didn’t increase the platelet count. 59 Pts were observed because the platelet count was greater than 30x10^9/L without bleeding symptoms. 148 Pts were treated; their mean platelet count at diagnosis was 25 (range 0-118); their mean age was 45 (range 4-87). 58 pts had a platelet count <1x10^10/L and significant bleeding symptoms. All pts received steroids as the initial treatment. 134 pts were treated within 3 months from diagnosis, while 14 were treated 4-156 months after diagnosis. After steroid therapy, 70 pts (47.2%) achieved a complete remission, 56 pts (37.8%) a partial remission, while 18 pts (12%) were refractory; 4 pts were not evaluable. 68 pts among the 148 treated pts (46%) received a second-line therapy; mean distance from first to second therapy was 54.5 months (range 1-225). Their mean age was 47.5 (range 4-85). Second-line therapies were: splenectomy (18), azathioprine (7), vincu alkaldoids (18), cyclophosphamide (9), alf-lFN-2 (2), steroid (18) and high dose immunoglobulin alone (7); 5 more pts received high dose immunoglobulin associated with other therapies. 23 Pts were splenectomized after one (15 Pts) or more (8 Pts) medical treatments. At splenectomy the median age was 35.5 (range 11-76); mean platelet count at diagnosis in this group of pts was 21.4 (0-118). Mean time from diagnosis to splenectomy was 22.1 months (range 1-95). 15 pts (62.5%) achieved a CR, 7 (30.4%) a PR, 1 (4%) was refractory. Two patient developed deep thrombosis after surgery. We conclude that steroid therapy is effective as the initial treatment in most pts, while splenectomy was effective in most pts and could be performed without significant toxicity.
PO-200

RITUXIMAB TREATMENT IN ADULT IMMUNE THROMBOCYTOPENIC PURPURA


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Introduction. Immune Thrombocytopenic Purpura (ITP) is an autoimmune disease results from accelerated platelet destruction by autoantibodies to platelet glycoproteins. Therapeutic options are: steroids, intravenous Immunoglobulins, anti-D, splenectomy. More difficult cases of chronic ITP may require additional therapy with danazol, azathioprine, cyclophosphamide, vincristine and alkaldoids. Patients and Methods. In the last few years Rituximab, a chimeric murine/human anti CD20 monoclonal antibody that depletes B cells, has been used extensively in B-cell lymphoma and various autoimmune diseases, including adult and childhood ITP. We refer our experience about 10 cases of refractory adult ITP, treated with Rituximab, administered as a weekly I.V. infusion of 375 mg/m2 for 4 consecutive weeks. In responders the administration of rituximab was continued at the same dosage for 4 bimonthly infusions. Inclusion criteria were relapse of ITP, after conventional therapy, with platelets <50 x 10^9/L). Three patients showed Complete Remission (plts >150x10^9/L); two patients reached Partial Remission (plts between 50 and 150x10^9/L). So Overall Response was obtained in 5 patients (50%). Five patients had no response (plts<50x10^9/L). Two patients experienced mild or moderate infusional reactions, easily surmounted. Conclusions. Rituximab is an effective and safe option for the treatment of adults with relapsed ITP without serious reactions. In the future Rituximab may have a role in the initial management of ITP. It may be useful working out prognostic factors that predict response in eligible patients and studying long-term effects of Rituximab.

PO-201

NON-HODGKIN LYMPHOMA (NHL) AND DEEP VENOUS THROMBOSIS: A DANGEROUS LIASON STILL UNEXPLORED


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Introduction. DVT incidence ranges from 3 to 15% in cancer patient. Nevertheless there are few data about DVT in lymphoma. Aim of our study is to define the real DVT risk and incidence in lymphoma patient. Methods. Our study is a dicentric retrospective study. We considered age of patients, sex, histological type of lymphoma (low grade -LG- vs high grade -HG), localization over or under diaphragm, extranodal localization, vascular compression, stage of disease, NHL, LDH level, chemotherapy type and duration, type of lymphoma, patients with NHL and ITP. We also evaluated the presence of vWF deficiency, platelet dysfunction, fibrinogen levels. Results. 87 patients (15% showed DVT. Of these, 37(43%) were localized at legs and 19(22%) regarded abdominal veins (especially iliac veins, 10% of total). DVT onset median time was 3 months from NHL diagnosis (R0-156 months). Sex, histological type of lymphoma (LG vs HL), localization over or under diaphragm, extranodal localization, stage of disease, IPI, LDH level, chemotherapy type and use of chemotherapy regimen containing methotrexate were not related to an increased risk to develop DVT. Vascular compression was the most risk factor for DVT development with OR 3.7 (CI95%: 1.8-7.3), Chi Square 13.4 (p<0.0001), positive predictive value 0.71(CI95%: 0.57-0.82), specificity 0.95(CI95%: 0.92-0.97). Discussion. In our study vascular compression by enlarged lymphnodes, patient age ≥60 y.o., and weekly administration of chemotherapy seem to be the main risk factors to develop DVT in NHL patients. The DVT development within 3 months from NHL diagnosis seems to be a risk factor for non response/relapse.

PO-202

EARLY PLASMA-EXCHANGE EXCHANGE RESULTS AS THE BEST THERAPEUTIC CHOICE IN THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)/HEMOLYTIC UREMIC SYNDROME (HUS): A SINGLE INSTITUTION EXPERIENCE


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Introduction. It is known that Plasma Exchange (PE) allows 80% of remission rates in TTP patients, while there is no agreement as yet on the efficiency of therapies with corticoids, anti-aggregants, immunosuppressors and anti-CD20 (Rituximab) antibodies. In this study we retrospectively evaluated the efficacy of PE in TTP pts treated in our Center since March 1998 to January 2007. Methods. Fifteen pts (5 males and 10 females), mean age 58 years (9-62) were treated. Clinical presentation: 60% had neurological symptoms; 30% had acute renal failure. Mean Plts was 27x10^9 micro/L (3-100), Mean Hb 7.0 g/dl (4.0-10.0), Mean LDH 2423 U/dL (637-4000), Mean reticulocytes 8% (3-7). All patients immediately received therapy sessions with PE (OCTAPLAS-KEDRION) using KOB E SPECTRA apparatus; mean sessions 7.4 (2-16); 14 (93%) pts received acetylsalicylic acid, 10 (66%) corticosteroids, 7 (47%) Vincristine and 1 (7%) high-dose immunoglobulins. Results. Eleven (73%) pts showed a haemostatical response with a complete disappearance of neurological symptoms and renal failure; Plts reached >100x10^9/µL, and Hb > 10 gr/dl was reached with an average of 6 (2-10) and 9 (4-21) PE sessions, respectively. Six (40%) pts showed a hypocalcemia, 4 (27%) hypokalemia and 2 (13%) hypotension. None of them had fever, infections or PT alterations. One (6.7%) patient resulted refractory to the first 8 PE courses and achieved complete remission after 4 Vincristine courses, high-dose immunoglobulins and new 24 PE. Three (20%) pts died precociously because of disease progression. Out of 11 pts in remission, 2 pts had relapse and were recovered with new PE sessions: a third patient, who relapsed for 3 times, resulted refractory to plasma-to-plasma exchange, Vincristine and Cyclophosphamide: after the demonstration of ADAMTS 13 activity reduction and anti ADAMTS 13 antibodies level increase, 4 Rituximab courses administration allowed a complete remission lasting since 2 years. At present all the 12 pts are still in complete remission. Discussion. Our experience confirms that PE treatment in TTP is the most effective and safe in inducing remission of both haematological and related symptoms. The side effects linked to this treatment often are weak and immediately reversible in absence of mortality. Further studies are required in order to evaluate Rituximab therapy in TTP patients with recurrent relapses, with reduced ADAMTS 13 activity and/or anti ADAMTS 13 antibodies increase.

PO-203

THE EFFICACY OF RECOMBINANT FACTOR VILLE (NOVOSEVEN) IN A PATIENT WITH SEVERE FACTOR XI DEFICIENCY AND ABNORMAL BLEEDING


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Factor XI (FIX) deficiency is an inherited autosomal recessive disorder associated with bleeding of variable severity. Individuals with factor XI deficiency may need specific therapy for surgery, accidents and dental extractions. Several therapies are available which include fresh frozen plasma (FFP), factor XI concentrates (Haemoven, LFB, France), fibrin glue, antifibrinolytic drugs, desmopressin and possibly recombinant Factor VIII (Novoseven, Novo Nordisk). We report the case of a 22-year-old chemotherapy: OR 3.7 (CI95%: 1.8-7.3), Chi Square 13.4 (p<0.0001), positive predictive value 0.71(CI95%: 0.57-0.82), specificity 0.95(CI95%: 0.92-0.97). Discussion. In our study vascular compression by enlarged lymphnodes, patient age ≥60 y.o., and weekly administration of chemotherapy seem to be the main risk factors to develop DVT in NHL patients. The DVT development within 3 months from NHL diagnosis seems to be a risk factor for non response/relapse.
old female with a severe congenital FXI deficiency (FXI level <1%) admitted to our hospital for full term delivery. Before the delivery, a prophylactic 20 ml/kg FFP treatment was given to reach a factor XI target of 50% or more. Because of unexpected obstetrical complications, urgent Caesarian section was required and despite large volume infusion, FFP was unable to establish an adequate post-operative haemostasis. The post-surgical course was complicated by severe bleeding from surgical sites, requiring adequate supportive transfusional therapy. On day 10, due to a septic complication, a disseminated intravascular coagulation appeared and the clinical haemorrhagic picture further worsened. FFP therapy was stopped and FXI concentrate at dosage of 15 U/kg was given to achieve an adequate haemostasis, with improvement but not bleeding disappearance. Despite the Haemoleven therapy, at day 20, the patient became epileptic and a cerebral CT revealed a cerebral haemorrhage. Therefore, she received a course of four Novoseven infusions (the first at 90 micrograms/kg) followed by three 4 hour interval infusions at 15 micrograms/kg with complete resolution of the clinical picture and no side effects or evidence of thrombosis. Three weeks later the cerebral CT images revealed the complete disappearance of the haemorrhage and the substitutive therapy with FXI concentrate was discontinued. In our experience the use of recombinant FVIIa was able to ensure adequate haemostasis in a patient with severe factor XI deficiency and abnormal bleeding.

**PO-204**

**RECOMBINANT FVII ACTIVATED PROVIDES EFFECTIVE BLEEDING CONTROL IN POST-PARTUM ACQUIRED HEMOPHILIA: DESCRIPTION OF A CASE WITH UNUSUAL CLINICAL PRESENTATION**

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**Background.** Acquired hemophilia A (AH), which is often life-threatening, is a rare hemorrhagic disorder caused by auto antibodies directed against coagulation FVIII in patients with no family or personal history of bleeding diatheses. In contrast with bleeding in joints and muscles typical in hereditary hemophilia, common bleeding sites are skin, mucosa, muscles and retroperitoneum. **Aim.** We report a case of post-partum AH which unusually presented right knee hemarthrosis, successfully treated with recombinant FVII activated (rFVIIa) and prednisone. **Case Report:** A 40-year-old woman, who had given birth to a baby two months before, referred to our centre with right knee hemarthrosis and several soft tissue hematomas in the upper and lower limbs. The post-partum vaginal bleeding was not excessive. Laboratory investigation revealed a prolonged APTT (70 sec.; ratio 2.27) not corrected by the addition of normal plasma and anemia (Hb 11 g/dl). A rhamnotic screening was negative. These clinical and laboratory data raised the suspect of the presence of a FVIII inhibitor. Recombinant FVIIa was started [99 micrograms intravenously (i.v.) every 3 h]. Concomitant immunosuppressive therapy with prednisone (1 mg/kg) was prednisone (1 mg/kg) was started. At the same time, hematuria arose worsening anemia (Hb 8.5 g/dl). On post-recovery day 3, the patient was found to have FVIII activity <1% and a FVIII inhibitor titre of 11 B.U and FVIII activity was in the normal range in the coagulation tests. Over the next 4 weeks. At this time, the FVIII inhibitor was not measurable and FVIII activity was in the normal range in the coagulation tests. **Conclusions.** The clinical presentation of AH in this case was atypical. Joint bleeding is an unusual clinical manifestation of post-partum AH. Immunosuppressive therapy was mandatory with prednisone being the first line inhibitor eradication treatment. Our case has documented the effectiveness and safety of rFVIIa in stopping bleeding in a woman with post-partum AH.
Comparisons among assays and standards were performed using an ANOVA model for repeated measures. We confirm previous results about a better sensitivity of the one stage method for the lowest concentrations of F VIII, with a more accurate evaluation of terminal half life. Measured Cmax is slightly superior to expected values and independent from the assay used. The clinical utility of RLS in the evaluation of FVIII concentration after infusion of BDD-rFVIII seems to be lower after reformulation of the product.

| Table 1. Phenotype and genotype in five patients with severe FXIII deficiency. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Chromogenic Assay | Reflecto ST | Plasma ST | One stage assay | Reflecto ST | Plasma ST |
| Mean | SD | Mean | SD | Mean | SD | Mean | SD | p |
| r2 | 0.98 | 0.99 | 0.97 | 0.99 |
| Cmax (0/mL) | 0.63 | 0.14 | 0.63 | 0.09 | 0.60 | 0.13 | 0.58 | 0.13 | ns |
| Tmax (minutes) | 10.4 | 2.8 | 10.3 | 2.7 | 10.4 | 2.8 | 10.5 | 2.9 | <0.05 |
| Half-life (hour) | 9.4 | 3.6 | 9.7 | 4.3 | 10.4 | 2.8 | 10.9 | 4.7 | <0.05 |
| AUC inf | 575.0 | 147.0 | 575.0 | 147.0 | 575.0 | 147.0 | 575.0 | 147.0 | <0.05 |

**PO-207**

**PLASMA VEGF LEVELS CORRELATED WITH MALIGNANCY GRADE IN CANINE MAST CELL TUMOUR MODEL**

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Introduction. Some experimental and clinical data strongly suggest that angiogenesis is associated with haematological malignancies and that increase in microvascular density (MVD) is associated with increase in mast cell density. Canine mast cell tumour (MCT) is common in dog with an incidence much higher than that found in human and MCT share several biological and clinical characteristics with human mastocytosis. Several reports suggest that mast cells may play a role in neovascularization by several pro-angiogenic factors such as Vascular Endothelial Growth Factor (VEGF) stored in their secretory granules, furthermore it has been suggested that MVD and mast cell density are significantly correlated in MCT progression. To evaluate the role of plasma VEGF concentrations in progression of this tumour model and to correlate VEGF concentrations and MVD, a series of 63 MCT has been investigated.

Methods. Venous blood was obtained from a series of dogs with clinical diagnosis of MCT. Blood sample was dispensed into a sodium citrate, theophylline, adenosine, dipyrindamole tubes for plasma (P) (Becton Dickinson Hemogard Vacutainer Systems, Plymouth, UK). P samples were centrifuged at 180 x g x 10 min. The supernatant of P was removed obtaining a platelet-rich plasma (P-RP). P-RP was treated with thrombin (80 microlitri/mL) obtaining a plasma activated platelet rich P-APR. VEGF levels were examined in P-APR using the Quantikine Human VEGF ELSA R&D Systems Inc., Minneapolis, MN. From the same cases formalin-fixed paraffin embedded cutaneous lesions were obtained. Definitive histological diagnosis was performed on haematoxylin and eosin and Undritz method (Merck, Darmstadt, Germany) and 68 cases with MCT were selected. Cytohistological grade of tumour differentiation was done according to the classification of Fatsaik and there were (25 G1, 19 G2, 24 G3 cases). For the evaluation of MVD a three-layer technique was adopted. As a canine pan-endothelial marker the rabbit polyclonal antibody anti FVIII-RA was employed. The ten most vascular area hot spot were selected and individual vessels were counted at X400 fields. Results. Mean value standard deviation (s.d.) of VEGF levels in P-APR were: 670 240 s.d., 325 199 s.d., and 287 188 s.d. in G1, G2 and G3 MCT respectively. Differences in mean VEGF concentrations in G3 MCT vs G1 MCT and G3 MCT vs G2 MCT were significantly (p=0.002 and p=0.004 respectively by t-test). A significantly correlation between mean VEGF concentrations and MVD was demonstrated (p=0.001 in G1, p=0.002 in G2, p=0.001 in G3 MCT by Pearson test). Discussion. In this reports we found that the levels of VEGF in P-APR correlated with MVD and malignancy grade of canine MCT model. Our data indicated that mast cells of G3 MCT contain few cytoplasmic granules, on the contrary mast cells of G1-G2 MCT were less degranulated. This morphologic pattern may be the expression of a higher release of VEGF in G3 as compared to G1 and G2 MCT. Accordingly G3 MCT were higher vascularised and had a higher circulating VEGF and a potential consequence of this angiogenetic activity is the higher incidence of metastases in this sub-group. Taken toher these data suggest that angiogenesis and metastatic potential are associated with high malignancy grade MCT and that mast cells actively participate in this process through the realese of VEGF contained in their secretory granules.

**PO-208**

**VENOUS THROMBOEMBOLISM IN PATIENTS WITH ACUTE LEUKEMIA**


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Venous thromboembolisms (VTE) may occur in patients with cancer and cause substantial morbidity and mortality. Although there are several published data on the association of VTE and malignant diseases little is still known about patients with acute leukaemia (AL). Medical records of all patients with AL treated in our institution between November 2001 and November 2006 were reviewed. Patients with Acute Promyelocytic Leukemia and full-blown Disseminated Intravascular Coagulation (DIC) were excluded from the analysis. A total of 67 patients with AL were eligible for analysis (36 M and 31 F; median age 55 years, r: 25-80 years). 15 out of 67 patients had at least one VTE. Venous thromboembolisms included central venous catheter-associated venous thrombosis in 6 patients and 9 non-central venous catheter-associated VTE. Interestingly, by analysing immunophenotypic pattern of leukemic blasts at the diagnosis, we found in 15 out of 15 patients the expression of CD54 (ICAM-1) and CD49d (VLA-4) at high density. We used Enoxaparin for all the patients. The therapy was thus scheduled: Deep Venous Thrombosis (DVT): patients received 200 iu of enoxaparin/Kg/day until resolution of acute symptoms (generally fifth-seventh day), an halved dose for 30 days and further halved was prescribed for the following 60 days. For Pulmonary Embolisms (PE) patients starting doses were 400 iu/kg/day; halved doses after clinical resolution (10-15 days) for 30 days; a further halved dose was proposed for at least 5 G3 MCT were higher vascularised and had a higher circulating venous central catheter (VCC) received 4000 iu daily until the removal of catheter, when infusional need ceased. VTE appears to be a common complication of ALs, mostly during chemotherapy, and sometime is really threatening. Anticoagulant or antithrombotic therapies are problematic because of the high incidence of contemporary thrombocytopenia or bleedings. In our hands Enoxaparin seems to be effective and safe, not inducing thrombocytopenia, not influencing the trend of minor bleedings. The over-expression of certain adhesion molecules, such as ICAM-1 and VLA-4, on leukemic cells may play a role as thromboembolic risk factor. We conclude that it is mandatory to assess every and each factor of thrombembolic risk at the same time of the diagnosis of leukemia in order to start with a prompt and adequate profilaxis.

**PO-209**

**USE OF RITUXIMAB IN AUTOIMMUNE THROMBOCYTOPENIA OF CHILDHOOD. DESCRIPTION OF A CASE**

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Background. We describe a case of a paediatric patient with idiopathic thrombocytopenic purpura (ITP), relapsed after immunosuppressant therapy, successfully treated with rituximab. Case report. A twelve years old patient suffering for severe ITP from January 2003, formerly treated with different treatments (steroids, immunoglobulins), comes to our Poster
attention for relapse in July 2005. Because of evident signs of steroid’s side-effects and no response to treatments (PLT=35.000/mm³), we decided for immunotherapy with rituximab (375 mg/m² once a week for eight doses, then further four doses once a month), obtaining normalization of platelet’s count. Laboratory tests are normal after a follow-up of 22 months. No adverse episode to refer; reduction of IgG and IgM has been moderate, although this event is commonly described. Discussion. ITTP in childhood is usually self-limiting disease, resolving shortly. In 10% of cases, thrombocytopeny persists after six months. In the event of hemorrhagic manifestations or/and platelets values particularly low, the treatment consists in the steroidal and immunoglobulin therapy; splenectomy is not advisable for infective complications. That treatments, occasionally protracted for years, are complicated by serious adverse events, yet. Rituximab has been successfully given to adult patients with autoimmune disorders (Zaza E. et al. 1 Exp Hematol. 2006 May 84(5):571-2; Gottenberg E. et al. Annals of Rheumatic Disease 2005 64:913-920), but experience in childhood is still poor (Parodi et al. Int. J. Hematology 2006 July; 84(1):48-5). Conclusions. Use of antibody, anti-CD20, as treatment of ITP of childhood, represents an important therapeutic way-out, also considering the absence of known side-effects.

**PO-210**

**VENOUS THROMBOEMBOLISM IN COLORECTAL CANCER PATIENTS**


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Background. Cancer patients are at greater risk of Deep Venous Thrombosis (DVT) and death compared to non cancer patients. About 6% to 20% of patients with DVT are cancer patients. Cancer associated thrombosis is the second leading cause of death in oncologic patients. Few data are available regarding incidence of DVT in colorectal cancer patients. Aims. To describe the incidence and outcomes of colorectal cancer patients with DVT. Patients and Methods. A retrospective analysis of 51 colorectal cancer (23 left colon cancer, 14 right colon cancer and 14 rectal cancer) from September 2003 to March 2007 was performed. Median age was 68 (range 48-81), 19 (37%) were women and 45 patients (86%) underwent major surgery. 23 (45%) were stage IV, 12 (23.5%) stage III and 16 (32%) stage I-II. Statistical analysis were performed by Yates corrected Chi-square test, Odds Ratio (OR) and Relative Risk (RR). Survival analysis was performed by Kaplan Meier methods. Results. 7 patients (13.7%) had a symptomatic DVT event, of these, 6 patients (86%) had stage IV cancer, 1 patient stage II rectal cancer with DVT event present at diagnosis. Among 23 patients with stage IV disease, 6 of them (26%) were diagnosed with a DVT event. In patients with metastatic disease, Yates corrected Chi-square test was 2.6 (p=0.051) with an OR of 8 (CI 10.01-54.7) and RR of 6.3 (CI 1.0-40.5). Overall survival (OS) was 18 months in patients with DVT event and 20 months in patients without DVT events (p=0.3). DVT survival no significant difference in survival were observed among patients with stage VI disease with or without DVT events. Conclusions. DVT is more frequent in metastatic disease. In a larger cohort of patients we need to confirm if OS is not influenced by accounting of DVT event in metastatic colorectal cancer patients.

**PO-211**

**PERIOPERATIVE USE OF 1-DEAMINO (8-D-ARGININE) VASOPRESSIN (DDAVP) IN A PATIENT WITH BERNARD SOULIER SYNDROME**

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Bernard-Soulier Syndrome (BSS) is a rare hereditary bleeding disorder with autosomice recessive transmission characterized by prolonged bleeding time, thrombocytopenia, and giant platelets. The underlying defect is a deficiency or dysfunction of the glycoprotein GPⅡb-Ⅲa complex, a platelet-restricted multsubunit receptor required for normal primary hemostasis (its role consists in binding the von Willebrand factor). Genes coding for the four subunits of the receptor, GPⅡb, GPⅡa, GPⅣ and GPⅥ, map to chromosomes 17p12, 22q11.2, 3p29, and 3q21, respectively. It is well known that 1-deamino (8-D-arginine) vasopressin (DDAVP) can reduce the bleeding time in patients with BSS. We report the case of a 58 year old female patient with an homozygous BSS and dysfunction of the glycoprotein GPⅡb-Ⅲa complex. She presented in poor clinical conditions, with jaundice, purpura, gingival hemorrhage, anemia and thrombocytopenia. Ultrasonography of abdomen showed gallstones, some obstructing terminal choledochus. Soon she required urgent endoscopic sphincterotomy (ES). As the bleeding time was unevaluable and she had massive blood losses, we administered dDAVP 0.4mcg/Kg six hours before sphincterotomy. Bleeding time shortened to ten minutes and blood loss was reduced. The patient received two single-donor apheresis platelets immediately before the procedure and did not show relevant bleeding. In the following days we observed a transient hyperamylasemia and progressive reduction of bilirubin levels. At discontinuation of dDAVP (four days after sphincterotomy) she had a gastroenteric hemorrhage and received red cell concentrates, platelets and two more doses of dDAVP. Clinical conditions improved and bilirubin and amylase levels normalized. Our experience confirms that dDAVP can improve hemostasis in patients with BSS and thus allow surgical procedures due to the significant reduction of the hemorrhagic risk. It is still unclear how to establish the number of doses needed to avoid either tachyphylaxis or late hemorrhages once the drug has been discontinued.

**PO-212**

**PERI-PARTUM ACQUIRED HAEMOPHILIA IN A 36-YEARS OLD WOMAN: MANAGEMENT OF POST-PARTUM CRITICAL BLEEDING AND ERADICATION OF THE INHIBITOR.**

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Acquired haemophilia A is a rare bleeding disorder, with an incidence of one-two new cases per million of the population per year. It is caused by the development of specific autoantibodies which neutralize the procoagulant activity of factor VIII. Overall, post-partum factor VIII inhibitors constitute 7 to 21% of the cases of acquired haemophilia A but when they do occur, within a few days after delivery, they are frequently associated with severe, life-threatening uterine bleeding. We report on the management of a 36-years old primigravida who developed a peripartum inhibitor against factor VIII. Although the APTT was prolonged at caesarean delivery, the acquired coagulopathy remained undiagnosed. The patient manifested 9 days later a significant vaginal bleeding which became rapidly life-threatening and required the transfusion of large amounts of packed red cells. At that time, the presence of a low-titre (4 BU/mL) factor VIII inhibitor was recognized and treatment with activated recombinant factor VIII was started. However, the persistence of severe bleeding and sepsis induced the gynaecologists to proceed to hysterectomy. The wound of the uterus appeared infected and dehiscent at visual inspection during surgery. In spite of a peri-surgical satisfactory haemostasis, obtained through the use of the same bypassing agent, an acute anaemia occurred in the following 24 hours, requiring a careful surgical revision of the pelvis. Afterwards, the clinical conditions of the patient improved greatly but the post-operative course was further complicated by infection, dehiscence of the surgical wound and urinary bladder rupture. The inhibitor did not disappear spontaneously in the following days and was managed by intravenous massive therapy with steroid and cyclophosphamide. Outcome was favourable in this patient. However, it should be noted that an earlier diagnosis of acquired haemophilia in this woman could have allowed to better manage the post-partum haemorrhage and to avoid surgical complications.
PO-213  
**ASSESSMENT OF THROMBOTIC RISK IN CANCER PATIENT: PROPOSAL OF A SCORING SYSTEM.** A MONOCENTRIC STUDY ON 119 PATIENTS

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**Background.** Deep venous thrombosis (DVT) is present in about 3-15% of cancer patient. Paraneoplastic thrombosis pathogenesis is multifactorial. Anti-thrombotic prophylaxis frequently is not oriented to real thrombotic or haemorrhagic risk of patient. **Aims.** Aim of our study is to define the real thrombotic risk in neoplastic patient. With this purpose we considered in our patient complement fraction C3 and C4 and immune circulating complex (ICC) because they activate macrophage and platelets and increase tissue factor level. Moreover we investigated also total cholesterol and triglycerides level, because they are linked with factor VII activation. **Methods.** We considered C3, C4, ICC, cholesterol and triglycerides level in 119 patients with solid neoplasm (62colon, 27lung, 18gastric, 12 others) and without anticoagulant prophylaxis. Of these only 102 were evaluable because in these complete data were available. Median age was 68.5 years (R 57-83). M/F ratio was 72/47. The threshold value of third quartile was chosen as risk cut-off (C3: 190 mg/dL; C4: 32 mg/dL; ICC: 2.9 mcg/mL; total cholesterol: 205 mg/dL; triglycerides 123 mg/dL). We elaborate a scoring system in which 1 point was attributed to each value inferior to third quartile. The statistical analysis was conducted with Yates corrected chi square test, Odds Ratio (OR), relative risk (RR). **Results.** 19 patients (16%) showed DVT. Of these 14 (74%) had a score ≤5 and 5 (24%) patients (81%) did not show DVT. Of these 50 (60%) had a score ≥24 and 26 of 33. Yates corrected chi square test is 5.36 (p 0.015), with an OR of 4.2 (95%CI 1.4-12.3) and a RR of 3.2 (95%CI 1.3-8.3). Negative predictive value is 0.91 (95%CI 0.83-0.96) and positive predictive value is 0.30 (95%CI 0.21-0.35). Sensitivity was 0.73 (95%CI 0.53-0.87) and specificity was 0.60 (95%CI 0.55-0.63). The statistical analysis was conducted with Yates corrected chi square test, Odds Ratio (OR), relative risk (RR). **Results.** 19 patients (16%) showed DVT. Of these 14 (74%) had a score ≤5 and 5 (24%) patients (81%) did not show DVT. Of these 50 (60%) had a score ≥24 and 26 of 33. Yates corrected chi square test is 5.36 (p 0.015), with an OR of 4.2 (95%CI 1.4-12.3) and a RR of 3.2 (95%CI 1.3-8.3). Negative predictive value is 0.91 (95%CI 0.83-0.96) and positive predictive value is 0.30 (95%CI 0.21-0.35). Sensitivity was 0.73 (95%CI 0.53-0.87) and specificity was 0.60 (95%CI 0.55-0.63). **Summary/Conclusions.** Neoplastic patient frequently shows haemorrhagic risk (eg in chemotherapy induced thrombocytopenia). Therefore anti-thrombotic prophylaxis could be useful for the effective and haemorrhagic risk of neoplastic patient. Our scoring system is useful in distinguishing cancer patient with low thrombotic risk and consent to avoid anti-thrombotic prophylaxis in patient with higher bleeding and lower thrombotic risk. Nevertheless these data need confirmation on a larger cohort of patients.

Po-214  
**ANTIPHOSPHOLIPID SYNDROME (APS): A CASE REPORT OF LEG BILATERAL ARTERIAL AND VENOUS THROMBOSIS IN A 48 YEARS OLD MAN**

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Antiphospholipid syndrome (APS) is a systemic disorder characterized by arterial and/or venous thrombosis, fetal loss, thrombocytopenia and presence of antiphospholipid antibodies (APA) or LA positivity. For APS diagnosis we need at least 1 clinical criterion and 1 laboratory criterion. We want to report a case of APS with arterial and venous thrombosis and presence of APAs. On 2005, October a 48 years old man demonstrated right leg subacute ischemia with evidence by vascular ultrasonography (US) of bilateral arterial thrombosis (iliac-femoral on the right and femoral on the left) plus iliac-femoral venous thrombosis on the right leg. He underwent thromboendarterectomy on bilateral femoral arteries and venous thrombectomy, after retrievable cava filter placement. Platelets count was 107.000-140.000/mm3 in the days after surgery. Oral anticoagulation therapy (OAT) was started four days after surgery. Laboratory assay demonstrated: LA positivity; anticardiolipin antibody (Ab) (ACA) IgG 85 GPL/mL (normal range 0-10), ACA IgM 7.7 GPL/mL (normal range 0-7); Beta2Glicoprotein1 Ab (Beta2GP1 Ab) IgG: 34 UI/mL (normal range <1), Beta2GP1 Ab IgM: 9 UI/mL (normal range <1). No other thrombophilic alterations were demonstrated (Factor V Leiden, Prothrombin 20210 mutation, homocysteine, protein C, protein S and ATIII), nor dyslipidemic disorders were present and platelets number was normal. Four months later, during OAT, the laboratory findings were confirmed (LA positivity; ACA IgG 177 GPL/mL, ACA IgM 4.2 GPL/mL; Beta2GP1 Ab IgG: 251 UI/mL, Beta2GP1 Ab IgM: 9.1 UI/mL and we decided not to remove now the cava filter. On 2006, July the patients stopped OAT in order to avoid any interference on laboratory assay, and under low molecular weight heparin therapy (100 UI/Kg x 2 day), LA and APAs tests were performed. All the alterations were confirmed: LA positivity; ACA IgG: 168 GPL/mL, ACA IgM: 6.2 GPL/mL; Beta2GP1 Ab IgG: 743 UI/mL, Beta2GP1 Ab IgM: 6.3 UI/mL. Together with the vascular surgeon we decided not to remove the cava filter. On 2007, April the patients underwent a US control with the evidence of e worsening of arterial stenosis both in right and in left leg (occlusion of external iliac artery on the right leg and critical stenosis of common femoral artery on the left). The situation was confirmed by arteriography but in absence of symptoms, there was not indication for intervention by the vascular surgeon. OAT during this period was well conducted and INR always in therapeutic range (2,5-3) so that we decided to add to OAT low dose acetylsalicylic acid (ASA 100 mg/day). Last laboratory assay on 2007, April are: ACA IgG: 175 GPL/mL, ACA IgM: 3 GPL/mL, Beta2GP1 Ab IgG: 9 UI/mL, Beta2GP1 Ab IgM: <1 UI/mL; LA not performed.

PO-215  
**RITUXIMAB AS SALVAGE THERAPY FOR REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA**

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**Introduction.** Idiopathic thrombocytopenic purpura (ITP) is an immune disorder with a tendency to become chronic in adult patients. After failure of first line corticosteroids in patients requiring therapy, a wide variety of treatments, such as splenectomy or immunosuppressive agents, have been used with different results. Recently, Rituximab (RTX) has been proven as effective in different autoimmune diseases, including ITP. **Methods.** We report our experience in a cohort of 17 patients with relapsed or refractory ITP which were treated with RTX 375 mg/sqm weekly for 4 cycles. There were 3 males and 14 females with a median age of 35 years (19-62). All patients were confirmed as having ITP with ASH criteria (George et al., 1996), and had been diagnosed a median of 35 months before first RTX infusion. Written informed consent was obtained. Previous treatments included in all patients prednisone and in 8/17 patients different immunosuppressive drugs (e.g. IVIg, vincristine and azathioprine). Furthermore, 4 patients had undergone splenectomy. When starting RTX, 14/17 cases were still receiving low/standard dose of prednisone, which was tapered and stopped within 2-4 weeks after first dose. **Results.** Eight patients obtained a complete remission (CR) of ITP defined as a stable platelet (Plt) count over 100×10^9/L, while 7 patients reached confirmed Plt count between 50 and 100, registered as partial remission (PR), with an overall response rate of 88%. The 2 patients refractory to RTX were treated with splenectomy (result: CR) and with a further course of low dose RTX after two months (result: unsustained PR), respectively. Time to achieve Plt ≥50 was variable, median being 21 days (7-60). Toxicity due to RTX was mild and was observed only at first infusion in just 5 cases [2 laryngeal edema (grade 2), 2 cutaneous rash (grade 1), 1 headache (grade 1)]. No significant hematologic toxicity or infections were reported during therapy or subsequent observation. After a median follow-up of 17 months (1-24), 13/15 responders as in continuous remission, while 2 relapses were observed at 3 and 12 months in a PR and a CR patient, respectively. **Discussion.** We confirm the efficacy of RTX in patients with refractory ITP, with a high response rate and negligible toxicity; time to response is variable, ranging from 1 week to 2 months. Our data, along with those from literature, candidate RTX as ideal salvage therapy. The optimal schedule remains to be established.
MYELOMA AND MONOCLONAL GAMMPATHIES I

PO-216
LOW RISK IGG MONOCLONAL GAMMPATHY OF UNDETERMINED SIGNIFICANCE (MGUS): PROPOSAL AND VALIDATION OF A PROGNOSTIC SCORING SYSTEM

Rossi F, Marcheselli L, Rossi D, Callea V, Guffanti A, Federico V, De Munro M, Baraldi A, Musto P, Bacigalupo A, Gaidano G, Petrucci MT, Goldaniga M, De Paoli L, Baldini L, 1UO Ematologia 1, Ospedale Maggiore Policlinico MaRe, Università degli Studi di Milano, 2Divisione di Ematologia e Ematologia, Università degli Studi di Modena e Reggio Emilia, Modena; 3Divisione di Ematologia, Università del Piemonte Orientale A. Avogadro, Novara; 4Divisione di Ematologia, Azie- da Ospedaliera Bianchi-Melacrinio-Morelli, Reggio Calabria, 5Divisione Med-icina I, Ospedale Fatebenefratelli e Oofalino, Milano; 6Divisione di Ematologi- a, Università La Sa- piera, Roma; 7Divisione di Ematologia, Università Campus Bio-Medico, Roma; 8Divisione di Ematologia, Azienda Ospedaliera Nazionale SS Antonio e Baggio C. Aringo, Alesandranlla, 9CROB, Rionero in Vultur- e; 10Divisione di Ematologia II, Ospedale San Martino, Genova, Italy

We previously described in a series of 386 patients (pts) with MGUS (Blood:87:3,912,1996) a subset of IgG MGUS at low risk of evolution in multiple myeloma (MM) on the basis of the following simple hematological variables: serum MC 1.5 g/dL, absence of Bence-Jones proteinuria (B) and normal serum polyclonal Ig levels. For this group of pts, a non-invasive diagnostic approach (without skeletal radiology and bone mar- row evaluation) and a less close follow-up (FU) could be proposed. Our actual study analyzes two MGUS patient populations: a study sample, which is the extension of our previous series (553 pts, M/F ratio: 0.98, median age 61 yrs, range20-87 and a test sample (378 pts, M/F ratio: 0.96, median age 62 yrs, range 23-89) which is a group of pts referring to other Italian Hematological Institutions. The goals of our report are to evaluate the long-term evolution of study sample, to validate the low-risk variables on the two different populations and create a scoring sys- tem useful for clinical prognostic stratification. The median FU was 6.8 yrs (1.0-27.8) in the study group vs 5.7 yrs (1.247) in test group; in the first 47/553 (8.4%) pts developed MM vs 22/378 (5.8%) in the latter. At multivariate statistical analysis, the low-riskVariables previously defined, confirmed their statistical significance in the study sample: MC 1.5 g/dL (p<0.001), absence of B proteinuria (p=0.009), normal polyclonal Ig lev- els (p=0.002); furthermore, also age > 70 yrs resulted strongly related to MM evolution. Assigning a score equal to 1 to each variable related to progression (MC>1.5, presence of B proteinuria, reduced polyclonal Ig, age >70 years), we created a scoring system able to stratify the patients into different subsets with significantly different 10 yrs-evolution risk: low risk (score =0); HR 1.0, intermediate risk (score =1); HR 4.25 and high risk (score =2); HR 12.16. This scoring system was also validated in the test sample, even if the percentage of cases presenting low-risk variables was significantly lower and the median follow-up was shorter. Our data sup- poses the existence of low-risk IgG MGUS, in such cases a non-invasive diagnostic approach and less intensive monitoring planning are justified. Moreover we have identified a scoring system useful for the clinical prognostic stratification of MGUS patients.

PO-217
TRANSCRIPTIONAL FEATURES OF MULTIPLE MYELOMA PATIENTS WITH CHROMOSOME 1q GAIN


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Introduction. Abnormalities of chromosome 1 are among the most frequent chromosomal alterations in MM (45% of patients). The long arm of chromosome 1 is associated with amplification (1q/gain) that can occur as isochromosomes, duplications or jumping translocations. 1q/gain MM patients are characterized by complex karyotypes and aggressive disease, and their number increases as the condition goes from smoldering to overt MM, suggesting that these regions contain critical genes for disease progression. These findings along with the limited information concerning specific transcriptional profiles, prompted us to molecularly characterize 1q/gain MMs by FISH and microarray analyses. Methods. Purified plasma cells from 77 MM at diagnosis were characterized by FISH for the presence of 11 polysomy, the most recur- rent IGH translocations, ploidy status, chromosome 13 deletion, and by global gene expression profiling using the Affymetrix U133A arrays. Assessment of 1q gain by FISH was performed by using three BAC clones specific for the BCL9 (1q21.1), CK51B (1q22) and ARF1 (1q42.3) loci, and setting the threshold as 10%. Results. 1q/gain was identified in 47/77 (59.1%) patients; three (75%) or four (12.5%) signals of all the 1q probes were found in 35 patients and, in the remaining five samples (12.5%), the probes mapping to 1q21 and 1q22 showed more signals than that mapping to 1q42. 1q/gain was observed in the majority of purified plasma cells (median 96%) in all but three patients (range 12- 20%). 1q extra copies significantly correlated with chromosome 1 dele- tion (p<0.04), and the absence of chromosome 11 polysomy (p=0.038), but not with ploidy status (p=0.097). The differential expression of 61 genes (mainly localized on chromosome 1q12-1q44) distinguishes MM patients with or without 1q/gain. Functional analysis of the identified genes revealed their involvement in energy production pathways, intracellular protein transport, and endoplasmic reticulum-stress induced responses. The transcriptional fingerprint was robustly validated on a publicly available gene expression dataset, with a global classification rate of 85.2% for the independent cohort of MM cases. Discussion. These data improve our knowledge concerning the specific genes/pathways deregulated by 1q abnormalities, and provide a promising focus for fur- ther studies aimed at defining new therapeutic strategies in MM.

PO-218
MELPHALAN, PREDNISONE AND THALIDOMIDE (MPT) VERSUS MELPHALAN AND PREDNISONE (MP) IN NEWLY DIAGNOSED MYELOMA: RESULTS OF A PROSPECTIVE, RANDOMIZED PHASE III STUDY


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Introduction. Since 1996, oral melphalan and prednisone (MP) has remained the treatment of choice for elderly pts. This multicentre ran- domised trial compared oral MP plus thalidomide (MP) with MP alone in 60 to 80 years old pts. Methods. Patients were randomly assigned to receive oral MP (N=129) or MP alone (N=126). Inclusion criteria were newly diagnosed multiple myeloma, age >65 years, Durie and Salmon stage II or III, measurable disease. Exclusion criteria were another can- cer, psychiatric disease and any grade 2 peripheral neuropathy. Patients in MP arm received oral MP (melphalan 4 mg/m2 and prednisone 40 mg/m2 for 7 days) for six 4-week cycles plus thalidomide (Pharmonit LTD, Windsor, UK) 100 mg per day continuously until any sign of relapse or progressive disease. No anticoagulation prophylaxis was adminis- tered until December 2003 when prophylaxis with enoxaparin at 40 mg per day for the first 4 MP cycles was introduced. Patients in MP arm received oral MP, as in MPT arm. The primary objectives were response rate and event-free survival (EFS). Secondary objectives included all survival (OS), prognostic factors and incidence of any grade 3-4 adverse event. Results. The response rate and the EFS were significantly improved with MPT compared with MP. The partial response rates were 76.0% for MP and 47.6% for MP alone, including nCR of 27.9% and

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BENEFITS OFFERED BY INCORPORATION OF THALIDOMIDE INTO UP-FRONT DOUBLE AUTOGRAFT TRANSPLANTATION FOR MULTIPLE MYELOMA

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The Bologna 2002 study introduced thalidomide and dexamethasone (thal-dex) into up-front double autologous stem-cell transplantation (ASCT) with melphalan 200 mg/m² (MEL-200) for multiple myeloma (MM). That (200 mg/d) and dexamethasone (40 mg/d x 4 d every month) were administered from the output until the day before the second ASCT. Aim of the present analysis was to assess whether addition of thal-dex to double ASCT improved the outcome in comparison with double autotransplantation not including thal-dex. For this purpose, we performed a retrospective, case-match comparison of 138 patients enrolled in the Bologna 2002 study with an equal number of pair mates who were randomly assigned to receive double ASCT in the Bologna 96 trial. In comparison with the Bologna 96 study, patients enrolled in the Bologna 96 trial did not receive thal-dex; in addition, they were treated with VAD as induction therapy and with melphalan plus busulfan in preparation for the second ASCT. Case-matching was performed with respect to age, serum β2-microglobulin level and clinical stage. The completion rate of double transplantation was 64% in the Bologna 96 study and 60% in the Bologna 2002 trial (p=0.7). On an intention-to-treat basis and using the International uniform response criteria, the probability to attain a very good partial response (VGPR) and complete response (CR) after the different treatment phases was higher for patients enrolled in the Bologna 2002 (thal+) study compared with the Bologna 96 (thal-) trial (p<.003 for induction therapy; p<.001 and =.001 for first and second ASCT, respectively). As a result of the better quality of response assured by incorporation of thal-dex into MEL-200-based double ASCT (69% vs 49% for the control group), both relapse-free survival (RFS) and event-free survival (EFS) were significantly longer in the Bologna 2002 study compared with the Bologna 96 trial (5-year projected RFS: 41% vs. 38%, respectively; p=0.005) (5-year projected EFS: 45% vs. 25%, respectively; p=0.01). Administration of novel agents for treating post-transplantation relapses likely minimized a potential survival gain offered by the addition of thalidomide (5-year overall survival rate: 70% vs 54% for the control group; p=0.20). It is concluded that incorporation of thal-dex into MEL-200-based double ASCT significantly improved the rate of response and extended both RFS and EFS in newly diagnosed MM patients.
PO-224
LONG TERM RESPONSE AND SURVIVAL AFTER THALIDOMIDE IN RELEASED/ REFRACTORY MULTIPLE MYELOMA
Zapposodi P,1 Corso A,1 Barbarano L,1 Brasca P1, Petrucci MT,1 Calabrese E,1 Palumbo A,1 Brighen S,1 Pascutto C,1 Mangiacavalli S,1 Varettoni M,1 Pica GM,1 Lazzanino M1
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Introduction. The advent of thalidomide (thal) has radically changed multiple myeloma (MM) patients’ (pts) outcome. It has proven to be effective at low doses either in released/refractory disease or at onset, better if in combination with steroids. However, few studies have provided long term results of thal therapy. Aim of this retrospective multi-center study was to evaluate long term efficacy and toxicity in 303 MM pts treated with thal alone or with steroids. Methods. A total of 303 MM pts (median age: 63 years) diagnosed in four Italian Centers were treated with thal with/without steroids (251/72) after a median of one previous line of therapy (1-6) including high dose Melphalan in 126 pts. Remissions were categorized as Complete Response (CR), partial remission (PR), negative immunofixation, <5% bone marrow plasma cells, Very Good Partial Response (VGPR) (positive immunofixation, decrease in the M-protein ≥90%), Partial Response (PR) (decrease in the M-protein ≥50%), Stable Disease (SD) (decrease in the M-protein 25-49%), No Response (NR) (decrease in the M-protein <25%). Median follow up was 18.8 months (1-85). Results. Thal was administered at a median daily dose of 100 mg (50-600) for a median period of 12.6 months (mo). The best response, obtained after a median of 5.3 mo (0.3-37) since the start of thal was assessed in 291 pts: CR+VGPR: 35 (12%), PR: 130 (45%), SD 43 (15%), NR 83 (28%). Median time to progression was 14 mo with significant differences according to type of response (p=0.000): 23 mo in CR+VGPR, 15 mo in PR, 12 mo in SD and 6 mo in NR group. Progression Free Survival was 20.6 mo with statistical difference in response subgroups (p=0.000): 35 mo in CR+VGPR, 21 mo in PR, 19 mo in SD and 8 mo in NR. Interestingly, comparison between SD and PR curves did not show any significant difference (p=0.3). Overall survival was 26.2 mo with statistical difference in response subgroups (p=0.000): 63 mo in CR+VGPR, 31.5 mo in PR, 25 mo in SD and 10 mo in NR group. Concerning toxicity the most clinically relevant complications were: neuropathy in 119 pts (40%), constipation in 80 pts (26%), and thromboembolic events (TVP or pulmonary oedema) in 21 pts (7%). At the time of analysis, thal was reduced in 81 pts (36.5%) and discontinued in 128 pts (66.6%) because of disease progression and of toxicity in 70% and 30% of cases respectively. Conclusions. Thal produces high response rate in released/refractory MM pts with better results in pts with responses of good quality. However, even those pts achieving a SD have a long period of remission (19 mo), not significantly different from that of pts obtaining a PR. Neurotoxicity remains the main complication of the treatment.

PO-222
THE ORAL PKC-BETA INHIBITOR ENZASTAURIN (LY317615) INHIBITS PROLIFERATION AND INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELL LINES
Verdelli D,1 Nobili L,2 Todoerti K,1 Lombardi L,1 Marmiroli S,3 Civailleri M,1 Cosenza M,1 Bertacchini J,1 Albright D,1 G suppressing tyrosine kinase inhibitors was strongly correlated with that generated on the gene expression profiles of the patients in the dataset. Furthermore, we identified different genes with altered expression level corresponding to local copy number variations. Discussion. Our results showed that genomic structural abnormalities in multiple myeloma closely reflected in expression imbalances. Our data reinforce the importance of using novel high-throughput approaches to provide insights into the characterization of novel potential genetic lesion in primary myeloma tumors.

PO-225
PERCUTANEOUS FEMORALPLOMOSPLASTY, ILIOPLASTY AND SACROPLASTY IN THE TREATMENT OF BONE LYTIC LESIONS IN ADVANCED MULTIPLE MYELOMA
Pusceddu C, Ballicu N, Sabiu D,1 Carubelli A,1 Podda G, Angelucci E1 U.O. Radiologia Ospedale Oncologico A. Busino, Cagliari; 1U.O. Ematologia e Centro Trapianti Midollo Osseo, Ospedale Oncologico A. Busino, Cagliari, Italy

Aim. to evaluate feasibility, safety and efficacy of femoroplasty, ilioplasty and sacroplasty under Fluoroscopic and computed tomography (CT) guidance in the treatment of pelvis and femoral Multiple Myeloma associated osteolitc lesions. Methods: seven patients (2 men and 5 women, mean age 68 years), affected by multiple bone. Myeloma localization, were treated by percutaneous bone-cement injection with polymethylmethacrylate solution (PMMA) into the supractetabular (5 cases), sacral (2 cases) and femoral bone cavity (2 cases). Lesion approach was performed using a 10-gauge bone biopsy needle under CT and Fluoroscopic control. Results: 122 lesions were selected under Fluoroscopic guidance. In a patient with large osteolytic defect in
the head and neck of right femur the tip of the needle was inserted whit percutaneous transtrochanteric approach. In a single case sacred painful fracture was reported since 8 months before. The procedures were performed using local anaesthesia and conscious sedation in all patients. Pain was measured with Huskisson visual analogue scale - VAS -(range 0 no pain – 10 maximum possible pain) before, 24 hours, 1, 3, 6 and 12 months after the procedure. RESULT: Technical success was achieved in all patients. Immediate post-procedural control with CT did not show complications which methylmethacrylate leakage or bleedings phenom- ena along the insertion of the needle. No other complications were recorder. VAS score decrease from a median values of 7.5 (range 8-4) to a median value of 0 (range 0-1.5). In a median follow up of 11 months (range 2-27 months) none of the patients developed pathological frac- ture in the intervention site. While before the procedure all patients head a severely decreased walking capacity depending by orthopaedic tutors after the above reported follow up 6 of them fully recovered independent walking ability. Conclusions. Percutaneous osteoplasty of the pelvis and femora is feasible and safe in patients affected by Multiple Myelo- ma bone lesions. Methylmethacrylate injection is minimally invasive pro- cedure providing immediate pain relief. Furthermore it contributes to reinforce of bone structure with prevention of pathological fractures and improvement of walking ability and finally quality of life.

PO-226
B-FGF, IL-6, TNF-ALPHA AND VEGF SERUM LEVELS IN NEWLY DIAGNOSIS MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE, DEXAMETHASONE AND AUTOLOGOUS TRANSPLANTATION

Cellini C,1 Tazzari V,1 Grafone T,1 Conti M,1 Baccini C,1 Zamagni E,1 Cangini D,1 Tacchetti P,1 Tosi P,1 Zaccaria A,1 Cavo M1
1UO Ematologia Ospedale Santa Maria Delle Croci Di Ravenna; Laboratorio Di Tumoriologica Ospedale Santa Maria Delle Croci Di Ravenna; Istituto Di Ematologia Ed Oncologia Medica I.E.A. Seragnoli Bologna, Italy

The aims of the present study were to investigate the relationship between serum concentrations of angiogenic cytokines and both baseline characteristics and response to therapy in a series of 96 patients (pts), previously untreated, with symptomatic multiple myeloma (MM) who were enrolled in the Bologna 2002 clinical study. All pts received four months of induction therapy with thalidomide and dexamethasone (thal-dex) in preparation for subsequent autologous transplan- tation(Tx). Serum levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) were measured at baseline and after ther- apy. For this purpose, laboratory assays were performed using quanti- tative sandwich ELISA. At baseline, serum levels of bFGF, IL-6 and TNF-α were significantly higher in MM pts than in healthy controls (p<0.0001, p=0.04 and p=0.0019, respectively). We found that serum levels of IL-6 were closely related with both TNF-α and VEGF levels (p=0.0012 and p=0.0142); serum levels of VEGF were significantly correlated with platelet count (p=0.0015). After thal-dex, the analysis of the patients with baseline serum levels above lower limit of detection of the assay showed a significant reduction of serum levels of TNF-α and IL-6 (p=0.007 and p=0.006); on the contrary, we observed a significant increase in the levels of VEGF (p=0.0001). Additionally, after Tx_1 and Tx_2 (both with melphalan 200 mg/m²) in 40 and 39 pts we observed a progressive significant reduction of serum levels of TNF-α (p<0.002) and IL-6 (p=0.008); and a significant reduction of serum levels of bFGF (p=0.011). We confirmed the progressive increased trend of VEGF (p=0.001). There was no significant difference of cytokines serum lev- els in responder and non-responder pts (the non responders are a mini- mal percentage, RR=85%). Finally, elevated baseline serum levels of bFGF were predictors for OS in a Cox proportional hazards regression analysis (p=0.0235). In conclusion, our observation regarding to signifi- cant reduction of serum levels of bFGF, TNF-α and IL-6 after therapy in a relatively wide population subject to an homogenous treatment and to the positive impact of baseline serum levels of bFGF on the OS, needs further research in order to understand the role of angiogenesis and angiogenic cytokines in newly diagnosed MM, and more properly to clarify their relationship with primary thalidomide therapy.

PO-227
PREDICTION OF RESPONSE TO PRIMARY THERAPY WITH THALIDOMIDE-DEXAMETHASONE (THAL-DEX) FOR NEWLY DIAGNOSED MULTIPLE MYELOMA BY GENE EXPRESSION PROFILING (GEP)

Terragna C,1 Renzulli M,1 Remondini D,1 Tagliafico E,1 Roncaglia E,1 Tosi P,1 Zamagni E,1 Tacchetti P,1 Perrone G,1 Ceccolini M,1 Brioli A,1 Fallotti MC,1 Martellini G,1 Baccarani M,1 Cavo M1
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Introduction. The Bologna 2002 clinical trial has provided demonstration of the superiority of front-line thalidomide and dexamethasone (thal-dex) over VAD in terms of increased rate of response and magni- tude of tumour reduction moreover the rate of grade 3 or 4 neutropenia. The majority of the patients enrolled in this trial obtained a partial response (PR) after induction therapy, whereas a complete response (CR) was observed in 20% of the total patients population. We have thus adopted a Gene Expression Profiling (GEP) strategy in order to identify a signature able to differentiate patients who are more likely to obtain a CR after induc- tion therapy with thal-dex. Patients and Methods. Plasma cells obtained at diagnosis from 86 patients enrolled in the trial were used throughout this study. GEP was performed in 52 patients, using the Affymetrix microarray platform. Real-time PCR using micro-fluidic cards was per- formed in 56 patients. Criteria of response were those established by Blad et al. Results. Overall, 19 of the 88 patients (22%) who obtained at least a nCR after thal-dex were analyzed. In the first study-phase, a training set of 52 patients was used in order to identify a gene signature of 162 genes which was able to significantly separate patients with nCR from the others (p<0.05); among the genes included in the signature, a list of 31 allowing to predict the nCR was selected, by adopting a Near- est-Neighbours (NN) classifier. In the second study-phase, the predictive value of the 31 genes was validated on a test set of 56 patients by Real- time PCR. In an attempt to identify the most significant predictive genes, a final list of five genes was then generated, which allowed to predict attainment of nCR with 76% sensitivity and 75% specificity. Of interest, the five-gene signature encompasses CCND1 and CCND2, as well as the anti-apoptotic gene CFLAR. Conclusions. These results are the first step to create a custom array or to adopt microfluidic cards using a small number of genes in an attempt to predict at diagnosis good quality responses to primary therapy with thal-dex in MM. Acknowledgments. Supported by Università di Bologna, Progetti di Ricerca ex-60% (M.C.); Ministero dell’Università e Ricerca Scientifica (MIUR), progetto FIBB, RBAU01269A_001 (M.C.); Fondazione Carisbo.

PO-228
A MULTICENTRE PHASE II TRIAL ON COMBINATION OF MELPHALAN, PREDNISONE, THALIDOMIDE AND DEFIBRITIDE IN ADVANCED STAGE MULTIPLE MYELOMA PATIENTS.

Larocca A,1 Rossi D,2 Pregno P,3 Masini L,1 Rus C,4 Magarotto V,1 D’Agostino E,1 Falco P,1 Gay F,1 Gugliotta L,1 Gaidano C,5 Jacobelli M,3 Mitiades C,4 Richardson PG,6 Anderson KC,6 Boccadoro M,1 Palumbo A1
1Divisione di Ematologia dell’Università di Torino, Azienda Ospedaliera S. Giovanni Battista, Ospedale Molinette, Torino, Italy; 2Divisione di Ematologia, Dipartimento di Scienze Mediche & IRCAD e Dipartimento d’Oncologia, Uni- versità degli Studi del Piemonte Orientale, Amedeo Avogadro, Novara, Italy; 3 Ematologia, Azienda Ospedaliera San Giovanni Battista, Torino, Italy; 4 Ematologia, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy; 5 Gentium S.p.A., Italy; 6 Jerome Lyper Multiple Myeloma Center Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Various studies have shown that Defibrotide (DF) possesses antithrombotic properties, without significant systemic anticoagulant effects and bleeding risk. This novel oligonucleotide has also demon- strated a remarkable activity in Multiple Myeloma (MM): DF is able to abrogate tumor cell interaction with marrow stromal cells and to increase in vivo (human MM xenografts in SCID/NOD mice) the respon- siveness of MM cells to melphalan, cyclophosphamide and dexametha- sone. We have investigated the impact of DF in association with Mel- phalan, Prednisone and Thalidomide (MPTD) and its protective role against thrombosis in relapsed/refractory advanced MM. A multicenter
phase I/II trial has been conducted to define the efficacy and safety of MPTD regimen. Safety was assessed by defining dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) of DF when administered in combination with MPT. DLT was defined by the occurrence, in >80% of patients at first cycle, of febrile neutropenia, or Grade 4 neutropenia > a week, or any other Grade 4 hematologic toxicity, or any > Grade 3 non-hematologic toxicity. MTD was the dose level prior to that resulting in DLT. We enrolled 24 patients between March and November 2006. Treatment schedule consisted of 6 cycles of oral melphalan (8mg/m2/day 1-4), prednisone (1.5mg/kg/day 1-4), thalidomide (50-100mg/day continuously) and DF at 3 different subsequent dose levels (17mg/Kg i.v. or 2.4 g p.o. D 1-4, 1.6 g p.o. D 5-35; 34mg/Kg i.v. or 4.8 g p.o. D 1-4, 3.2 g p.o. D 5-35; 51mg/Kg i.v. or 7.2 g p.o. D 1-4, 4.8 g p.o. D 5-35), repeated every 35 days. No prophylaxis against deep vein thrombosis (DVT) was administered. Toxicity and response were evaluated in 19 pts (median age 69 years-old) who completed at least one MPTD cycle. 5 pts were in first relapse and 4 in second relapse (primary refractory patients and/or patients receiving therapeutic anticoagulation were excluded). According to EBMT/IBMTR criteria, at least partial response was obtained in 42% of patients (including 16% very good partial response) after a median of 3 cycles and no difference in response rate was noted among the 3 DF dose levels, though follow-up remains short. Any cohort of the study reached MTD. DLT were considered unrelated to DF at dose level 1 and grade 3 in the 1st dose level, 1 acute myocar- dial infarction (AMI) in the 2nd and none in the 3rd. Toxicities ≥grade 3 consisted of neutropenia (47%), thrombocytopenia (10%), anemia (21%), whereas ≤5% of patients experienced non-hematological toxicities ≥grade 3. None patients experienced DTVs and no significant bleed- ing was reported. Treatment was interrupted in 3 patients for adverse events: AMI (because of additional anticoagulation required), ileus (because of the diagnosis of amyloidosis at AL and disease progression), and persistent G4 neutropenia in one heavily pre-treated patient. PharmacoKinetic studies and analysis of surrogates are ongoing. The study is currently ongoing and updated data will be presented at the meeting.

PO-229

MOLECULAR DEFINITION OF 17p11.2-p12 AMPLIFICATION IN MULTIPLE MYELOMA

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Introduction. Multiple Myeloma (MM) is a malignancy of clonal plasma cell characterized by a high genomic instability leading to structural and numerical alteration that increases with advanced phases of the dis- ease. Recently, a great progress into the definition of the marked genom- ical instability may be achievable through the integration of copy number and global gene expression profiling data. In this context our recent study on human myeloma has provided evidence that the rearranged regions of the 17p11.2-p12 region that we have identified are involved in the disease course. Early diagnosis is important to obtain best response and improve clinical outcome. aPBSCT might be safely be performed at expe- rienced transplant centres combined to neurological expertise.

Table.

<table>
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<tr>
<th>Day</th>
<th>OR (95% CI)</th>
<th>Median (95% CI)</th>
<th>RankSum</th>
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</tr>
<tr>
<td>3</td>
<td>0.52 (0.50, 1.06)</td>
<td>1.19 (1.13, 1.54)</td>
<td>.009</td>
</tr>
<tr>
<td>5</td>
<td>1.10 (0.84, 1.47)</td>
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<td>10</td>
<td>1.60 (1.34, 2.22)</td>
<td>2.80 (2.71, 3.41)</td>
<td>&lt;.0001</td>
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</tbody>
</table>

PO-230

ADVERSE PROGNOSTIC FACTOR OF P53 GENE DELETION DETECTED BY FISH IN MULTIPLE MYELOMA FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION

Pitini V, Arrigo C, Naro C, Siracusano L, Sciarrone F, Altavilla G
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In multiple myeloma the frequency of p53 deletions detected by fluo- rescence in situ hybridization (FISH) is reported to range from 9%to 34% and associated with poor survival. We investigated the relevance of p53 deletions to the clinical outcome of patients with multiple mye- loma (MM) treated with high-dose chemotherapy and autologous stem cell transplantation (ASCT). Material and Methods. Between January 1998...
and January 2004, 56 patients were diagnosed and treated for MM with high-dose chemotherapy followed by ASCT. All patients received 4 cycles of the VAD regimen (Vincristine, Adriamycin, and Dactinomycin) followed by stem cell mobilization with Cyclophosphamide 5 g/m² and granulocyte colony-stimulating factor and 1 course of melphalan 200 mg/m² immediately prior to ASCT. To detect p53 deletions, a Spectrum Red-labeled DNA probe (LSI p53 Vysis) specific for the p53 locus on 17p13.1 was combined with a Spectrum Green-labeled probe for the chromosome 17 alpha satellite-DNA centromere. Results. An interstitial 8p deletion identified by one red (p53) and 2 green (CEP17) signals was detected in 8 of 56 patients. The median percent of myeloma cells with an interstitial p53 deletion was 45% (range, 28%-90%). A FISH analysis informative for t(4;14),13q status and t(11;14) was available for all cases. Patients with p53 deletions had significantly higher serum calcium and creatinine levels, but there was no significant correlation between p53 status and C-reactive protein (CRP), albumin level and lytic bone lesions. The overall response rates were similar in patients with and without p53 deletions (65% versus 69%, respectively). However, patients with p53 deletions had significantly shorter PFS (median, 8.1 months) than patients without p53 deletions (median, 26.1 months). OS was also significantly shorter (median, 13.7 months) for patients with a p53 deletion than for patients without a deletion (median, 46.1 months). Conclusions. p53 deletions in pts with multiple myeloma treated with high-dose chemotherapy is an independent risk factor for both PFS and OS.

PO-233
REDUCED INTENSITY CONDITIONING REGIMEN FOR ALLOGRAFTING FOLLOWING AUTOGRAFTING HAS STRONG ANTI-MYELOMA ACTIVITY AND DELAYS DISEASE PROGRESSION COMPARED TO TANDEM AUTOGRAFTING
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Allografting is a possible curative approach for patients with advanced multiple myeloma (MM). Unfortunately this procedure is only an option for younger patients with HLA-identical siblings. Recently, nonmyeloablative regimens (RICCT) based on fludarabine demonstrate stable engraftment of allogeneic cells. High-dose therapy with stem cell transplant conditioning and novel targeted therapies represent two approaches to overcome resistance of multiple myeloma cells to conventional treatments. Autografting (AutoSCT) has been limited by high-relapse rates and conventional allografting (AlloSCT) by excessive transplant-related mortality (TRM) and toxicity. RICT is a less toxic procedure for AlloSCT that aims to exploit graft versus tumor effect, has been shown to achieve remissions in MM. High-dose therapy/AutoSCT followed shortly thereafter by RICT might improve outcomes in MM as compared to AutoSCT or conventional AlloSCT used alone. We evaluated toxicity, engraftment, chimerism, graft versus host disease (GVHD) and response to RICT after AutoSCT in 20 patients with advanced stage MM. We compared two retrospective cohorts of patients who underwent either tandem AutoSCT (HDT consisted of Melphalan 200 mg/m²) or AutoSCT followed closely by related RICT (patients with HLA-matched siblings). The two groups were matched for pre-transplant therapy, disease status at transplant, time from diagnosis to transplant. GVHD prophylaxis for RICT patients consisted of CyA/MTX. The major results are summarized in the Table. In the AutoSCT/RICT group the complete remission rate was higher and the risk of disease progression after transplant was reduced. All patients who reached CR responded after full chimerism and GVHD developed. This finding confirms the existence of a graft-versus-myeloma effect. Since the first clinical signs of response were noted between 70 and 120 days and max-imum response between 160 and 200 days after RICT (after DLI in one patient), these responses should be considered immunological responses. These data suggest that an autoSCT followed by RICT significantly reduces the incidence of disease progression.

PO-234
FLOW CYTOMETRIC (FC) EVALUATION OF THE BONE MARROW PLASMA CELL (BMPC) CONDITIONING REGIMES FOR RICT --- TBI/FLUDARABINE VS AUTOCA  --- TANDEM AUTOCA

Introduction. The addition of thalidomide and bortezomib to the standard oral melphalan/prednisone (VNMP) significantly increased response rate and prolonged progression-free survival (PFS) in advanced multiple myeloma (MM) patients (Palumbo et al., Blood 2007, 109(7):2767-2772). Subgroup analysis was performed to identify baseline parameters which may predict outcome after VNMP and to determine which patient subgroups most benefit from this drug combination. Methods. Thirty patients (median age 66 years) with relapsed or refractory MM after 1 or 2 lines of therapy, were treated with five 35-days courses of bortezomib (given at 3 dose levels: 1.0, 1.5 and 1.6 mg/m²) on days 1,4,15,22, plus melphalan (6 mg/m²) and prednisone (60 mg/m²) on days 1-5 and thalidomide (50 mg) on days 1-35. Some parameters such as age, beta2-microglobulin, albumin, haemoglobin, C-reactive protein, creatinine, chromosome 13 abnormalities, stage, bone marrow plasmacytosis, line of therapy and dosage of bortezomib were analysed in association with response rate and PFS, using chi-square and Cox model. Results. Twenty patients (67%) achieved a partial response, including 13 patients (45%) who achieved at least a very good partial response. The 1-year PFS was 61%, and the 1-year sur-vival from study entry was 84%. Subgroup analyses showed that only serum albumin < 3.5 mg/dl was loosely associated with a lower response rate (p=0.09); no statistical difference was noted between responses of the bone marrow plasma cells (BMPC) has been limited by high-relapse rates and conventional allografting (AlloSCT) by excessive transplant-related mortality (TRM) and toxicity. RICT is a less toxic procedure for AlloSCT that aims to exploit graft versus tumor effect, has been shown to achieve remissions in MM. High-dose therapy/AutoSCT followed shortly thereafter by RICT might improve outcomes in MM as compared to AutoSCT or conventional AlloSCT used alone. We evaluated toxicity, engraftment, chimerism, graft versus host disease (GVHD) and response to RICT after AutoSCT in 20 patients with advanced stage MM. We compared two retrospective cohorts of patients who underwent either tandem AutoSCT (HDT consisted of Melphalan 200 mg/m²) or AutoSCT followed closely by related RICT (patients with HLA-matched siblings). The two groups were matched for pre-transplant therapy, disease status at transplant, time from diagnosis to transplant. GVHD prophylaxis for RICT patients consisted of CyA/MTX. The major results are summarized in the Table. In the AutoSCT/RICT group the complete remission rate was higher and the risk of disease progression after transplant was reduced. All patients who reached CR responded after full chimerism and GVHD developed. This finding confirms the existence of a graft-versus-myeloma effect. Since the first clinical signs of response were noted between 70 and 120 days and maximum response between 160 and 200 days after RICT (after DLI in one patient), these responses should be considered immunological responses. These data suggest that an autoSCT followed by RICT significantly reduces the incidence of disease progression.

PO-234
FLOW CYTOMETRIC (FC) EVALUATION OF THE BONE MARROW PLASMA CELL (BMPC) CONDITIONING REGIMES FOR RICT --- TBI/FLUDARABINE VS AUTOCA  --- TANDEM AUTOCA

Table.

<table>
<thead>
<tr>
<th></th>
<th>Tandem AutoSCT (N=15)</th>
<th>AutoSCT + RICT (n=20)</th>
</tr>
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<tbody>
<tr>
<td>Age, median</td>
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<td>51 (range, 34-63)</td>
</tr>
<tr>
<td>Median number of prior cycles of Chemoth.</td>
<td>4 (range, 3-6)</td>
<td>4 (range, 3-6)</td>
</tr>
<tr>
<td>Time from Dx to 1st AutoSCT (median mo.)</td>
<td>6 (range, 5-60)</td>
<td>9 (range, 7-42)</td>
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<tr>
<td>Conditioning Regimen for AutoSCT</td>
<td>Melphalan (200 mg/m²)</td>
<td>Melphalan (200 mg/m²)</td>
</tr>
<tr>
<td>Conditioning Regimen for RICT</td>
<td>--</td>
<td>TBI/Fludarabine</td>
</tr>
<tr>
<td>Median days from AutoSCT to RICT</td>
<td>--</td>
<td>80</td>
</tr>
<tr>
<td>Complete Remission</td>
<td>14%</td>
<td>50%</td>
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<tr>
<td>Disease-Free Survival at 5 yrs</td>
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<td>30%</td>
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<td>Overall Survival at 5 yrs</td>
<td>28%</td>
<td>40%</td>
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compartment was evaluated by a gating strategy using a quadruple combination of CD38/S61/CD45 monoclonal antibodies in 140 BM samples (75 MM (51 crabMM and 24 sMM), 39 MGUS and 26 polyPL). Gene expression profiling (GEP) of BMPC from an independent panel was performed on U133A chips using the 7G scanner (Affymetrix). Results. A higher percentage of BMPC expressing CD19 were demonstrated in polyPL cases as compared with MM groups (p<0.0001), while a progressive increase of CD56 expression was found from polyPL to MM cases through an intermediate value observed in MGUS cases (<0.001). This was distinctly mimed 42% (AUC=0.920, p<0.0001) as the best cut-off value of CD19 expression to discriminate between polyPL and the remaining cases. In this regard, 92.3%, 33.3%, 0% and 3.9% of respectively polyPL, MGUS, sMM and crabMM were classified as having a high CD19 expression (CD19high). Analyzing the CD56 best cut off, 58% (AUC=0.745, p<0.0001) was the value to distinguish MM cases. The CD56high phenotype was detected in 0% of polyPL cases, in 64.7% of crabMM, in 62.5% of sMM and in 20.5% of MGUS. Combining CD19 and CD56 phenotypes, we divided cases in 3 groups, namely neoplastic CD19low/CD56high, normal CD19+/CD56+ and intermediate phenotype (ether CD56+/CD19+/ or CD19+/CD56-). The malignant phenotype CD56low/CD19high was detected in 0%, 14.3%, 26.8% and notably, the intermediate phenotype was identified in 7.7%, 46.2%, 64.7% of polyPL, MGUS, sMM and crabMM, respectively (p<0.0001). Phenotype correlated with pathological MRI signals of spine (p=0.058) and with the amount of serum monoclonal component (p<0.0001). A significant higher expression of the CD19 gene transcript was observed in normal subjects as compared with MGUS and MM. With regard to CD56, although its expression was higher in MM cases, it did not reach a statistical significance compared to MGUS or normal subjects. Conclusions. Normal/malignant PCs are distinguishable by flow and identify BMPC in patients with plasma cell dyscrasias. GEP provides similar results and support these findings. The combination ‘normal/neoplastic’ phenotype predicts the amount of serum MC and MRI alterations in MGUS and MM patients.

**PO-235 FACTORS AFFECTING OUTCOME IN MM PATIENTS TREATED WITH ANTRACYCLIN-THALIDOMIDE BASED THERAPY**

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Over the years heterogeneous outcome of multiple myeloma (MM) has been explained by several prognostic factors identified in patients receiving different therapies. Here we investigated the factors affecting response (≥ VGPR vs < VGPR), TTP and OS in 127 MM patients treated with ThaDD regimen (Blood, 2006). Median age was 71 (range 41-83 years), 66 were de novo and 61 relapsed/refractory MM, respectively. We analysed the following variables: age (≥70 vs <70), sex, ECOG performance status (0-1 vs 2-4), MM subtype (IgA vs others), D-S stage (I-II vs III), bone marrow plasmacytosis (≥ 80% vs > 80%), haemoglobin (< 11.0 g/dL vs ≥ 11.0 g/dL), platelets (< 150000 vs ≥ 150000/µL), B2-microglobulin (≥ 4.0 mg/L and stage 1 ISS achieved a significantly higher good quality response rate (≥ VGPR) if compared with those having higher values of the above parameters (85% vs 56%, p=0.025; 90% vs 60%, p=0.028; 87% vs 45.5%, p=0.003; 93.5% vs 61%, p=0.021, respectively). On the contrary standard monoclonal component, sFLC absolute value, bone marrow plasma cells, cytogenetics and therapy were not predictive of response. Moreover, patients with at least a 5-fold FLC decrease post 1-2 therapy courses achieved a significantly higher VGPR response rate (94.5% vs 57%; p=0.027). We observed a trend for longer TTP in patients with lower baseline FLCr values (NR vs 12.5 months, p=0.0974), with lower FLCr after therapy (18.2 vs 12.6 months, p=0.1154) and with lower baseline beta2-microglobulin value (60% vs 30%, p=0.025; 85% vs 60%, p=0.028, respectively). Our results suggest that baseline FLCr, as some other standard prognostic factors and not standard MC or sFLC absolute value, is predictive for response and TTP in MM patients treated with new drugs. It has to be yet confirmed whether a fast FLCr decrease after treatment can be an early predictor of MM outcome.

**PO-236 USEFULLNESS OF SERUM FLC ASSAY IN PREDICTING OUTCOME IN MM**

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Recent studies demonstrated that serum Free Light Chains (sFLC) level and ratio may be used not only as a close measure of tumor burden but also as a predictor of outcome in MM patients treated with conventional or high-dose therapy. We evaluated sFLC (FREELITE®, The Binding Site Ltd) and FL C ratio (FLC: pathological FLC/non pathological FLC) at baseline and after 1-2 cycles of therapy in 51 patients with MM. Twenty of them received thalidomide-based chemotherapy regimens while 11 were treated with bortezomib combined with chemotherapy. Median age was 66 years, 16 were de novo MM and 35 presented relapsed/refractory disease. Twenty seven were IgG, 12 IgA and 12 BJ MM, 49 patients had stage 2-3 ISS and median beta2-microglobulin was 4.0 mg/L (range 1.3-20 mg/L). At baseline median sFLC and FLCr were 1854 mg/L (range 50-18300 mg/L) and 106.5 (range 3-13000), respectively. Thirty-six patients (70.5%) had a response to treatment (≥ VGPR whereas 13 patients (25.5%) progressed during a median follow-up of 12 months. Patients with a baseline FLCr ≤ 60, beta2-microglobulin level ≤ 4.0 mg/L and stage 1 ISS achieved a significantly higher good quality response rate (≥ VGPR) vs < VGPR (p=0.003; 93.5% vs 61%, p=0.014), with beta2-microglobulin concentration ≤ 0.015) had a longer OS. As per TTP, univariate analysis showed that patients aged ≤70 years (p=0.014), with beta2-microglobulin concentration ≥ 3.5 µg/dL (p=0.038), serum albumin level > 3.5 mg/dL (p=0.008), normal serum CRP level (p=0.002), lower ISS (p=0.032), lower D-stage (p=0.072) and response to therapy ≥ VGPR (p=0.015) had a longer OS. The factors that remained significantly associated with a longer OS in multivariate analysis were normal sCRP level (p=0.002) and response to therapy ≥ VGPR (p=0.007). Normal sCRP level at enrollment is the only factor predicting response to thalidomide-anthracyclin based therapy. However, the final outcome is related either to sCRP level and to quality of response.

**PO-237 18F-FDG-PET/CT, 99mTc-MIBI AND MRI IN THE EVALUATION OF PATIENTS WITH MULTIPLE MYELOMA**

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The aim of our study was to compare 18F-FDG-PET/CT with 99mTc-MIBI and MRI of spine and pelvis in the evaluation of patients with multiple myeloma in order to assess the relative contribution of these imaging modalities in the staging of this neoplastic disease. Thirty-three newly diagnosed patients with multiple myeloma were studied. Diagnosis and staging of patients were made according to standard criteria. All patients underwent whole-body 18F-FDG-PET/CT, whole-body 99mTc-MIBI 41° Congress of the Italian Society of Hematology Bologna, Italy, October 14-17, 2007 haematologica/the hematology journal | 2007; 92(s3) | 141
MIBI and MRI of spine and pelvis within ten days and the results of these imaging studies were compared. ¹⁸F-FDG-PET/CT was positive in 32 patients (16 had focal uptake, 8 had diffuse uptake, and 15 had focal and diffuse uptake), ⁹⁹mTc-MIBI resulted positive in 30 patients (6 focal, 11 diffuse, 13 focal and diffuse) and MRI of spine and pelvis was positive in 27 patients (6 focal, 13 diffuse, 8 focal and diffuse). ¹⁸F-FDG-PET/CT showed a total of 196 focal lesions (178 in bone and 18 in soft tissues) 121 of which in districts other than spine and pelvis, whereas ⁹⁹mTc-MIBI visualized 63 focal lesions (60 in bones and 3 in soft tissues) 53 of which in districts other than spine and pelvis. In the spine and pelvis regions, ¹⁸F-FDG-PET/CT detected 75 focal lesions (35 in the spine and 40 in the pelvis), ⁹⁹mTc-MIBI visualized 10 focal lesions (1 in the spine and 9 in the pelvis) and MRI detected 51 focal lesions (40 in the spine and 11 in the pelvis). ¹⁸F-FDG-PET/CT showed to be more sensitive than ⁹⁹mTc-MIBI in the detection of focal lesions. Although ¹⁸F-FDG-PET/CT and ⁹⁹mTc-MIBI were more panoramic than MRI, MRI should be preferred for the detection of focal lesions in the spine.

**PO-238**

**¹⁸F-FDG-PET/CT IN THE EVALUATION OF BONE MARROW INFECTION IN PATIENTS WITH MULTIPLE MYELOMA**

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Recent studies suggest that ¹⁸F-FDG-PET/CT may be useful in the evaluation of patients with multiple myeloma (MM). However, while patterns of focal tracer uptake is frequently associated with symptomatic MM, the meaning of diffuse bone marrow tracer uptake is still unclear. The aim of our study was to evaluate the bone marrow uptake of FDG by maximum and average standardized uptake value (SUVmax and SUVavg respectively) and to correlate these parameters with clinical and hematological variables. Fifteen patients with newly diagnosed MM were studied. Diagnosis and staging were made according to standard criteria including hematological and biochemical work-up. All patients underwent whole-body ¹⁸F-FDG-PET/CT. Region of interest analysis was made on transaxial PET images of vertebral bodies of thoracic spine (from T6 to T12) and lumbar spine (from L1 to L5) and both average SUVmax and SUVavg were calculated. In the 15 patients studied SUVmax range was 1.42-4.18 while SUVavg range was 1.06-3.16. A statistically significant direct correlation was found between SUVmax and both percentage of infiltrating plasma cells (r=0.65, p<0.01) and amount of monoclonal component (r=0.53, p<0.05) while a significant inverse correlation was found between SUVmax and hemoglobin value (r=-0.67, p<0.01). Similarly, SUVavg was directly and significantly correlated with infiltrating plasma cells (r=0.65, p<0.01) and with monoclonal component (r=0.57, p<0.05) and was inversely and significantly correlated with hemoglobin level (r=-0.68, p<0.01). Semiquantitative bone marrow uptake of FDG may reflect plasma cells bone marrow infiltra-

**PO-239**

**OUR EXPERIENCE WITH BORTEZOMIB RECHALLENGE IN MULTIPLE MYELOMA**

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The role of bortezomib in advanced myeloma is well established, and its place in first line therapy is currently in evaluation. Not much is known yet about bortezomib efficacy in retreating patients affected by advanced multiple myeloma responsive to previous bortezomib courses. We treated 14 patients in such clinical conditions. Patients’ mean age was 72 years (r: 58-84) all receiving not less than three previous lines of treatment before being treated with bortezomib and dexamethasone. Twelve/14 patients had responded to this first bortezomib attempt (1 CR; >75% CM reduction in 4/12; >50% CM reduction in 5/12; <50% CM reduction in 2/12). Bortezomib had been interrupted for CM plateau in 4/12 or for adverse events (5/12 cardiac failure; 6/12 peripheral neuropathy; 8/12 bone pain). Two/14 patients were unresponsive. Mean time elapsed between first bortezomib employment and rechallenge was 9 months (r: 1-18). Treatment modality followed standard protocol (1.3 mg/mq days 1,3,8,11 every 21 days); in some cases course intervals were longer. Dexamethasone was added in all cases but one. Bortezomib rechallenge produced 9/14 partial response (>75% CM reduction in 2/14; >50% CM reduction in 5/14). Five /14 patients did not respond. Peripheral neuropathy with pain and diarrhoea were main adverse events: treatment interruption was not necessary, and dose reduction was sufficient to manage the events. Four/14 patients suffered from HZ reinfection. In conclusion, bortezomib can be administered again to myeloma patients responsive to previous bortezomib treatment with significant possibility of response and without significant risks.

**PO-240**

**ANGIOGENESIS IN PATIENTS WITH BISPHOSPHONATE-ASSOCIATED OSTEONECROSIS OF THE JAW**

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The use of intravenous administration of bisphosphonates is well established for the treatment of patients with metastatic bone disease, osteoporosis, and Paget’s disease. Bisphosphonates are generally well tolerated and associated with minimal adverse effects; however, osteonecrosis of the mandible or maxilla (ONJ) associated with the use of bisphosphonates is a newly described entity reported since the 2003. This was initially thought to be a rare condition, but over a short period of time, an increasing numbers of cases are been recognized. In a retrospective study in patients who had received intravenous bisphosphonates in 2003, ONJ was reported in 10.5%. Although the etiological process of ONJ seems to be multifactorial and several local factors (trauma, pathogens, immunodeficiency) could be relevant, two theories have been advanced to explain the mechanism for this complication. The leading theory suggests that ONJ is caused by cessation of bone remodeling and bone turnover by the basic osteoclast-inhibiting effect of these drugs. The second theory is based on experimental evidence showing that pamidronate and zoledronate also inhibit capillary neoangiogenesis and cause a significant decrease of circulating VEGF levels. To test the hypothesis that in patients with ONJ the bisphosphonates may interfere with endothelial cell proliferation, using direct three colors flow cytomteric analysis we evaluated the number of circulating endothelial progenitor cells in 8 patients with bisphosphonate treatment and osteonecrosis, 8 Multiple Myeloma (MM) patients with bisphosphonates treatment without ONJ and 5 normal subjects. We therefore characterized circulating endothelial progenitor cells (EPCs). They represent more immature cells, expressing the hematopoietic marker CD135, and their phenotype is CD34+133-VEGFR2. We also identified the more mature circulating endothelial cells (CECs) that lose CD135 and start to differentiate into mature endothelial cells. Their phenotype is CD34+ VEGFR2. MM patients showed an increase of CECs with respect the control subjects and ONJ subjects. EPCs and CECs were higher in MM patients compared to controls and ONJ patients. ONJ patients showed a decrease of EPCs compared to control subjects while CECs were similar to the controls group. Our results seem to show the possibility that bisphosphonates could have a antiangiogenic effect and a suppressive effect on circulating endothelial cells of patients with ONJ.
PO-241
BORTEZOMIB NEUROPATHY IN PATIENTS TREATED FOR MULTIPLE MYELOMA: A MULTICENTER EXPERIENCE.
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Introduction. Bortezomib (B) is the first proteasome inhibitor to be used in clinical practice, recently approved for second line treatment of multiple myeloma (MM) patients (pts). Several trials demonstrated that B is relatively well tolerated, causing manageable non-hematologic and hematologic toxicity. The principal dose limiting toxicity is represented by peripheral neuropathy (PN) that affected up to 35% of the patients. We retrospectively analyzed the incidence and severity of PN in 46 MM patients that received B, as single agent or in combination, at our institutions. Material and Methods. Patients characteristics were as follows: median age was 67 years (range: 42-77), 56.5% were men, 6 pts were at onset, and 40 pre-treated, median time from MM diagnosis to treatment with B was 21 months (range 0-111), β2-microglobulin 2.7 milligrams/L (1.6-12). Risk factors for PN included prior use of thalidomide (T) in 19 patients (41.3%), vincristine (VCR) in 26 patients (56.5%) and 4 patients (8%) had diabetes mellitus. Before treatment with B, 16 patients (34%) already had some form of PN <2 grade (NCI-CTC). Patients received B alone (n=5) or in combination with dexamethasone (n=41) and T (n=10) or chemotherapy (n=7). Results. Overall, the response rate (RR) was 75%. PN-2 was observed in 25 pts (54%), with grade 3-4 occurring in 28%. Median time to onset of B-related PN was 57 days (range,11-180) after B initiation. In most cases (n=28; 92%), patients had sensory symptoms, while 2 patients (8%) experienced both sensory and motor symptoms. B-related PN led to therapy discontinuation in 7 pts (15.2%). For PN treatment pts received mostly symptomatic therapy (analgesics, antiepileptics, antidepressants drugs and vitamin supplements). Of the 25 patients with B-related PN, resolution or PN improvement occurred in 15/22 evaluable pts (68%), at a median time of 85 days (range,28-300). Data analysis showed VCR and T pre-treatment as the strongest PN risk factors (p<0.04), while treatment with B-T association did not show PN worsening. Furthermore responses were independent to PN. Conclusions. PN is a relatively frequent side effect of B treatment, although it seems to be reversible in the majority of pts and manageable by dose reduction or discontinuation. Prior neurotoxic therapy represent the strongest PN risk factor. Further studies are warranted to better define the PN pathogenesis and optimal strategies for the B-related PN management.

PO-242
DAILY LOW DOSE THALIDOMIDE PLUS MONTHLY HIGH-DOSE DEXAMETHASONE AS CONSOLIDATION/MainteNANCE treatment in elderly multiple myeloma patients
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Background. Thalidomide (Thal) is currently considered as one of the most active anti-myeloma agents. Thal has shown significant activity as frontline, salvage and maintenance therapy as well as in patients with relapsed disease. The association of Thal with Dexamethasone (Dex) has shown a superior efficacy in de novo and relapsed MM patients. Aims. The purpose of this study was to assess feasibility, tolerability and efficacy of the Thal/Dex schedule administered as consolidation/maintenance therapy in elderly MM patients not eligible for aggressive therapy. Methods. A combination of Thal at dose of 100 mg/d with Dex at dose of 40 mg/d for 4 days every 28 days was administered to 31 MM patients. Median age was 71 years (range 47-83 years). Results. Before starting Thal and Dex schedule, 2 patients (6%) were in complete response (CR), 16 (52%) in partial response (PR) while the remaining 13 patients (42%) in stable disease (SD). After a median Thal/dex administration of 14 months (range 3-42 months), 2 patients (6%) discontinued treatment because of WHO grade III-IV peripheral neuropathy (1 pt) and severe fatigue (1 pt). Dose-limiting toxicities were also defined as WHO grade I/II constipation (12/31 pts), peripheral neuropathy (9/31), fatigue (8/31), dizziness and/or somnolence (9/31). Although no patient received anti-thrombotic prophylaxis, no case of DVT occurred during Thal/Dex therapy. Six out of 16 patients (58%) in PR and 10/13 (76%) in SD showed an improvement of the previous response. In particular, considering the best response after Thal/Dex therapy, 15 pts achieved CR, 13 PR, 2 PD and 1 SD. The overall response rate (CR and PR) has improved from 56% to 90% (overall CR from 7% to 50%). The median progression free-survival (PFS) and overall survival (OS) of the 30 patients treated with Thal/Dex were 13 months and 24 months, respectively. Conclusions. The results of this study indicate that Thal/Dex treatment, given as consolidation/maintenance treatment, may affect life expectancy of MM patients not eligible for aggressive treatment modalities.

PO-243
CHROMOSOMAL ABNORMALITIES IN MULTIPLE MYELOMA PATIENTS: IMPACT ON PROGNOSIS AND SURVIVAL
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The importance of genetic alterations and their prognostic impact is of growing interest in Multiple Myeloma (MM). Specifically independent studies have identified a poor prognosis associated with the presence of Immunoglobulin heavy chain translocations (t(4;14); t(14;16); t(14;20)) or deletion of chromosome 13. Furthermore, similar large studies have confirmed the favorable prognosis of t(11;14) and t(6;14) or hyperdiploidy in absence of chromosome 13 abnormalities or aneuploidy. In our Institution, 70 MM patients (60 at diagnosis, 7 at relapse and 3 during post-chemotherapy follow-up) were studied by conventional cytogenetic and FISH analysis. The mean age was 65.4 yrs (range 32-80), with male prevalence. In the majority of cases, the serum monoclonal component was IgG and IgA; 6 patients had light chain only, 1 patient had IgD and 1 IgG+IgM. At the time of analysis the stage were: I/II (n=3); III A (n=3) and III B (n=4); the mean bone marrow plasma cell concentration was 47.5% (range 6-100%). Cytogenetics and FISH analysis were performed simultaneously. An abnormal karyotype was detected in 25/35 successful culture: 2 pts showed del(13q), 1 pt monosomy of chromosome 13, 18 pts had an hyperdiploidy karyotype, 2 pts showed chromosome 1 abnormalities in hyperdiploid karyotype, 1 pt revealed del (1q) in a normal karyotype and 1 pt had hyperdiploid karyotype with 47 +iso(11q). FISH analysis, performed using the following commercial probes (LSID13S19 (13q14.3) and D13S25, LSI IgH/CCND1-XT, LSI IgH/FGFR3 and LSIp53 (Vysis)), was informative in all 70 pts. In all 3 pts carrying chromosome 13 abnormalities by conventional cytogenetics, FISH analysis confirmed del(13q). Moreover, del(13q) was found in 20 additional pts with normal, abnormal or non informative karyotype. The pts carrying an hyperdiploidy karyotype had the highest number of cells carrying del(13q). In our hands the analysis performed by LSI IgH/CCND1-XT (t(11;14)) and LSI IgH/FGFR3 (t(4;14)) probes showed only 2 positive pts each. The impact of chromosome 13 deletion on time to progression and overall survival was analyzed, only considering the most sensitive technique used (FISH). Both time to progression and overall survival showed a significant worse prognosis in del(13q) pts (p=0.034 and p=0.056 respectively). Our data, even thought obtained in a small series and only using FISH analysis, confirm the negative impact of chromosome 13 deletion on prognosis and survival of MM pts and show a lower frequency of t(11;14) than other reported series. This kind of genetic studies have a relevant role in discovering high risk MM pts and should be used in the choice of therapies and to design new clinical trials.
**PO-245**

**COMBINED ADMINISTRATION OF BORTEZOMIB AND DEXAMETHASONE IN THE TREATMENT OF REFRACTORY MULTIPLE MYELOMA (MM)**


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Multiple myeloma (MM) is a neoplastic disease especially affecting elderly patients even if in recent years it has been also observed in younger patients. The use of the proteasome inhibitor bortezomib has been recently introduced in the treatment of relapsed and/or refractory MM. In fact, Bortezomib has proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Bortezomib-based combination regimens have induced clinical benefits with manageable toxicities and may ultimately lead to improvement in the duration of response and survival of patients in the first-line setting. In our institution we are following 55 patients with stage II/III MM and 15 out of 55 (7 F and 8 M, median age: 71 years, r.: 67-80 years) stage II/III MM patients refusing thalidomide treatment after 6 cycles of Melphalan and Prednimusine regimen for excessive toxicity even if they presented progression disease (PD) at the clinical re-staging performed with both serum marker evaluation and cytological examination of bone marrow blood. All the 15 patients refused thalidomide treatment and underwent a treatment with bortezomib (1.3 mg/m² i.v. d. 1, 4, 8, 11 every 21 days) together with dexamethasone (40 mg i.v. d. 1, 4, 8, 11 every 21 days). At a clinical re-staging performed after four courses from the beginning of bortezomib-dexamethasone combined administration a partial remission (reduction of M-component > 50-75%) was recorded in 14 out of 15 patients while the remaining was in steady disease (SD). Thereafter all patients received further four courses of therapy. At one month from the end of treatment five of fifteen patients achieved a complete remission (negative immunofixation) and the remaining showed a partial remission (PR). At the present, (month + 19) only two patients show a progression disease, while four patients are in CR and nine in PR. Our results suggest that the combination of bortezomib and dexamethasone is effective and well tolerated in the treatment of refractory MM in elderly patients. Although there are several published data on the activity of the therapy based on the combination between bortezomib and dexamethasone, little is still known about the improvement in the duration of response and survival of elderly patients in the first or second line therapies.
The osteonecrosis of the jaw in patients treated with bisphophonates: Alessandra’s experience


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Background. Bisphosphonates (BIS), are strong osteoclast inhibitors that are used for osteoporosis and in the treatment of both solid tumors with bony metastasis and myeloma. There are growing reports of osteonecrosis of the jaw (ONJ) associated with the nitrogen containing bisphosphonates, Pamidronate and Zolendronic acid therapy. This study focuses on characteristics and frequency of ONJ in our institution. Methods. A multidisciplinary study group, for the ONJ, has been working, in hour Hospital, established by oncologists, radiologists, odontostomatologists and maxillofacial surgeons. We have written a local protocol, drawn from the most important international guidelines on ONJ correlated to BIS therapy. We selected all patients with myeloma who were treated with BIS over the last two years. These patients were submitted to a complete oral screening by dentist and to a jaw X-ray. When ONJ was suspected, a CT scan was made. We have also evaluated the presence of general risk factors (diabetes, alcohol abuse, coagulopathy) and type, dose and length of therapy with BIS. We have elaborated an internal model, in which every patient was assigned at one out three groups of risk: low, intermediate and high related to oral and parodontal situation. Results. Since November 2005 we have submitted 35 patients (12 males and 21 females) to screening for ONJ. The type of BIS used was: Pamidronate in 4, zolendronic acid in 18, pamidronate plus zolendronate in 14, alendronate + zolendronate 1, pamidronate + clo-dronate + zolendronate 1pz. The distribution of the patients in each group of local risk was: 18 (54.6%) in low; 12 (34.4%) in intermediate and finally 5 (9.1%) in high risk. Two patients out 35 (6%) presented ONJ. In both cases ONJ were present in maxilla. Both patients were treated with thalidomide and were edentulous with mobile dentures (intermediate risk). One patient was treated both with pamidronate (total dose 3165 mg) and alendronate (total dose 38 mg) and the second one, only with zolendronate (total dose 76 mg). Conclusions. We have found 6% of ONJ. Thought the numbers are small, this frequency seems consistent with the other experiences. Our analysis confirms an association between BIS therapy and the occurrence of ONJ. Additional risk factors may predispose the patients to the development of ONJ. Prospective more large studies are needed to determine which additional risk factors may predispose the patients to the development of ONJ.

Rituximab therapy in relapsed/refractory IgM related peripheral neuropathies Anti-MAG positive

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Monoclonal IgM-related peripheral neuropathies (IgM-PNP) constitute a rare and heterogeneous group of disorders, which are generally poorly responsive to treatment. In April 2006 we defined therapeutic protocol for the treatment of IgM-PNP anti-MAG (Myelin-Associated Glycoprotein) positive, unresponsive to standard immunomodulatory/immunosuppressive treatments (steroids, IVlg and azathioprine). The therapy consisted on Rituximab infusion at a standard dose of 750 mg/m² iv weekly for a consecutive 4 weeks. Primary endpoints were the efficacy (in terms of clinical and/or neurophysiological improving), toxicity and tolerability of the treatment and time to treatment failure. Here we describe the first three patients referred to our Institution. All patients were evaluated at diagnosis with a panel of laboratory tests, clinical and neurophysiological parameters (INCAT Disability Scale/Rankin scale, MRC scale, INCAT Sensory Sum Score; sensory and motor nerve conduction studies). Furthermore bone marrow biopsy and peripheral blood immunophenotypical analysis excluded in all patients an overt lymphoproliferative disease. After one, three, six and twelve months from therapy, patients were evaluated with complete neurological tests, serum anti-MAG antibodies and circulating blood B lymphocytes percentage. After the first course of Rituximab, 2 of our patients progressively improved their sensory loss and weakness reaching an independent gait after 6 months after treatment, persisting until now. Concomitantly with clinical improvement we observed a reduction of anti-MAG antibody titres; we did not find any changes in conduction velocity studies before and after treatment. The third patient didn’t reach any neurological improvement. After 1 year from the therapy, we detected in two responsive patients an increase of circulating anti-MAG antibodies concomitantly with initial weakness to the lower limbs during walking. These patients underwent to a second course of Rituximab. In conclusion, these preliminary data indicate that Rituximab may be effective in some patients with refractory anti-MAG IgM-PNP. Its efficacy and toxicity has to be verified in controlled clinical trials.

Efficacy and toxicity of contemporary intravenous treatment with Bortezomib, Melphanal and Dexamethasone in previously treated myeloma patients

Bortezomib, Melphanal, and Steroids are among the most effective drugs for treatment of multiple myeloma and the synergy of the combination has been demonstrated. However, so far the contemporary administration of these drugs has not been explored. We therefore designed a protocol with monthly courses of contemporary intravenous administration of these drugs in patients with advanced multiple myeloma. Bortezomib was given at dosage of 1.3 mg/m² i.v. days 1, 4, 8, 11, Melphanal 5 mg/m² i.v. days 1, 4, 8, 11, Dexamethasone 40 mg days 1-2, 4-5, 8-9, 11-12. So far, 21 patients have been enrolled. Median age was 64.5 (range 53-82). All patients had been already treated with a median of 2 previous lines of treatment (range 1-6). Seven patients had received autologous bone marrow transplant and five patients Bortezomib alone or in combination. All patients included in this study were no longer eligible for a bone marrow transplant procedure. Fourteen patients were resistant to previous therapies while 7 were considered as relapsed. Hematological toxicity grade 3 and 4 occurred in 38%, 36%, 33%, and 50% after I, II, III, and IV cycle respectively. Three patients developed also grade 3 non-hematological toxicity (Herpes zoster, vomit). So far, 4 patients have stopped treatment for toxicity after 1, 3, 5, and 4 courses. All of these patients were in stable disease. Five patients achieved a very good partial remission (M-protein not detectable at electrophoresis), 5 patients a partial remission (reduction of M-protein > 50%) while in two other patients reduction of the M-protein was associated with increase of bone marrow plasmacells. Two patients were in stable disease, one was in progression and 2 are not yet evaluated. In conclusion, the contemporary intravenous administration of Bortezomib, Melphanal, and Dexamethasone, appears to be an highly effective treatment even for heavily pretreated patients. However, in these patients, haematological toxicity appears to be too high and the dosage of the drugs or their schedule should be modified.

EVA in advanced multiple myeloma patients previously treated with thalidomide

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Bortezomib, a selective and reversible proteasome inhibitor, has shown its efficacy in patients with relapsed/refractory multiple myeloma particularly when combined with other agents. In this phase II study we assessed efficacy and safety of the EDA-V combination (etoposide, dexamethasone, cytosine arabinoside and bortezomib) in advanced MM patients previously treated with thalidomide. EDA-V regimen included etoposide 80 mg/m² iv on days 1-4; cytosine arabinoside 1 g/sm on day 5; bortezomib 1.3 mg/sm iv on days 1, 4, 8, 11 and dexamethasone 20 mg/m² iv on days 1-4, 4-8, 11.
mg days 1-2, 4-5, 8-9, 11-12. Non progressing patients received 6 cycles of EDA-V every 28 days as induction therapy followed by 3 cycles of bortezomib 1 mg/sm day 1, 8, 15 and dexamethasone 20 mg days 1-2, 8-9, 15-16 every 2 months (consolidation therapy) and prednisone 50 mg every other day (maintenance therapy). Actually 21 patients have been enrolled and all of them (13 M, 8 F; median age 67 years, range 47-82) are assessable for response and toxicity. Seven patients (33%) had refractory disease, 10 (48%) had received more than 2 lines of prior therapy (all patients had received thalidomide), 12 patients (57%) underwent previously 1 autologous stem cell transplant and 11 (52%) had a PS (WHO) ≥ 2. Overall response rate was 57%, a PR or better was achieved in 52% and a VGPR or better in 19% of patients. One year TTP, EFS and OS were 53%, 46% and 82%. In total, 57 cycles have been administered.

Non-hematological toxicities included ≥ grade 3 neurotoxicity in 5 patients (24%), grade 4 diarrhea in 1 (5%) and grade 4 cardiotoxicity in 1 (5%, one patient with acute heart failure). Grade 1-2 fatigue was observed in 9 (45%); no DVTs were reported but one patient developed stroke. Grade 3-4 thrombocytopenia were recorded in 4 patients (19%) while grade 3 neutropenia in 3 (14%). Grade 3 infections were observed in 2 patients (10%) and another patients developed VZV reactivation. In 5 patients (24%) bortezomib dosage was reduced because of neurotoxicity and 2 skipped dose for thrombocytopenia and icu. Five patients (24%) were dropped from protocol due to toxicity (i.e. 1 cardiotoxicity, 1 infection, 3 neurotoxicity). EDA-V has significant activity and manageable toxicity in advanced MM patients who had received prior thalidomide with a manageable toxicity.

**PO-251**

**FIRST LINE THERAPY OF PRIMARY PLASMA CELL LEUKEMIA WITH BORTEZOMIB**


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Plasma cell leukemia (PCL) is an aggressive, rare variant of multiple myeloma (MM) which represents about 2-4% of all MM. There are two forms of PCL: primary PCL (about 60% of cases) presents de novo in patients without previous evidence of MM, while secondary PCL, which accounts for the remaining 40%, consists of a leukaemic transformation occurring in about 1% of patients with a previously diagnosed MM. We have recently shown that bortezomib is an effective agent for the treatment of primary and secondary, heavily pre-treated PCL ([Musto et al, Cancer 2007, in press]). In the present study we focused on the effects of bortezomib as first line therapy in primary PCL. Six patients (three male, three female; 57 to 76 year-old) are so far evaluable. Circulating plasma cells ranged from 6 to 40x10^9/L. Median WBC count was 37x10^9/L (range 9-81). Three patients had concomitant extramedullary disease. Del 13 was observed in 3 out of 4 patients with available karyotype. Bortezomib was given using the standard schedule of 1.3 mg/sqm days 1, 4, 8, 11, with an interval of 10 days between cycles. One patient received dexamethasone and thalidomide, four doxorubicin and dexamethasone (PAD) and one oral melphalan and prednisone (MPV) in combination with bortezomib for 2-6 cycles. One patient underwent autologous stem cell transplantation after 4 PAD cycles. According to the International Uniform Response Criteria, four partial remissions (reduction of M-component > 50%) and two very good partial remission (disappearance of M-component at electrophoresis, but positive immunofixation) were achieved (100% overall response). All patients are alive after a mean follow-up of 9 months. Five out of them remain in remission phase, one developed progressive disease after 6 months. Grade 3-4 hematological toxicities and infections occurred in 4 patients. No other significant adverse effects were observed. Primary PCL is usually characterized by poor prognosis. Global response rate to standard chemotherapy is less than 50% and median survival is only 7 months. Stem cell transplantation may be more effective in some but not all patients. Our findings suggest that the front-line use of bortezomib in combination with other active drugs is very promising and could significantly improve the otherwise expected poor clinical outcome of primary PCL. Updated data about these and other not yet evaluable patients will be presented.

**PO-252**

**FIVE YEARS OF EXPERIENCE AND OBSERVATION WITH THERAPY OF MAINTENANCE WITH VERY LOW DOSE THALIDOMIDE AFTER AUTO-SCT IN MULTIPLE MYELOMA**

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New drugs and high dose therapy with auto-transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy, the limit of this drug is the toxicity dependent dose and this determines a poor compliance. It could be useful in the control of minimal residual disease. We used low dose of thal as maintenance after autologous transplantation in patient with MM from January 2002 and here we bring our experience after five years of observation. From January 2002 and here we bring our experience after five years of observation. From January 2002 to January 2007 15 patients (6 males and 7 females) with MM have been treated in our institution. Median age was 59; 5 years (range 48-72). 7 were IgG, 3 IgA, 3 light chains and 1 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 4/13 performed double auto-SCT. Three months after SCT these patients have begun the maintenance with thal 50 mg/die, to start thal maintenance 6 patients were in CR, 5 in PR and 4 resistant disease and the median somministration of thal has been of 12 months (range 3-24 months). Median follow up from the beginning of maintenance therapy was 42 months (range 15-60) with 8/14 (57%) patients in CR or stable disease, with a median progression free survival (PFS) of 45 months and overall survival (OS) projected at 61 months of 52% from to start thal. In our experience we have observed a neurological toxicity (grade I-II) in the 65% of the patients but only 4 have had to suspend the treatment; a haematological toxicity of grade I in the 55% of the patients that have not behaved interruption of the treatment and finally in any case we have documented thrombotic episodes.

**Figure 1.**

**Figure 2.**
Finally we have compared this group of patients with another group (14 patients) with the same clinical characteristics that we have observed in the same period but that have not effected maintenance with thal. In this last group 10/14 patients (71,5%) relapsed with median follow-up of 52 months (range 16-60) and median PFS and OS of 14 and 19 months respectively. The difference between the 2 groups is statistically significant for PFS (p=0.005), and not significant for OS (p=0.34) even if difference (62% vs. 0%) appears clear. (Figure 1-2). The median overall survival observed after progression, in the two groups, has been of 6,6 months in thal group and 7 months in the group of patients that have not effected the maintenance, this difference is not statistically different (p=0.06; Figure 3). In conclusion in 5 years of observation our experience has shown, even if the number of the patients is small, that maintenance with low doses of thal, after auto-transplantation, it not only has a good compliance but it improves the PFS and it doesn’t worsens the OS from the relapse.

Figure 3.

PO-253
AMYLOID IN BONE MARROW OF MULTIPLE MYELOMA
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Systemic AL amyloidosis is associated with about 15% of cases of multiple myeloma. Amyloid can be detected in bone marrow by Congo red staining as amorphous pink material or intracellular reddish spindle-shaped inclusion, recalling Auer rods. Bone marrow smears of 12 consecutive patients affected by multiple myeloma at diagnosis and 10 patients after treatment or relapse were stained by Congo red and studied by transmission and birefringence microscopy. Either focal or diffuse storages were considered positive. Overall, 11 patients resulted positive to Congo red and apple-green birefringence and 11 were negative. Out of 12 patients studied at diagnosis, 5 were positive: 4 of them were staged II or III, with IgGk (2pts.) or IgGlamba isotype (2 pts.); 1 was non secretory. Bone marrow plasma cells ranged from 28 to 68%. Out of the 10 patients with advanced disease, 6 were positive: 2 IgGk, 1IgAlamba, 2 poorly secretory (k and lambda, respectively). Bone marrow plasma cells ranged from 6 to 88%. It seems that amyloid deposition at microscopic level is a frequent occurrence in myeloma patients. By enlarging our cohort, we will investigate on possible relationship of amyloid with clinical and biological findings, as well as with treatment outcome.

PO-254
COMPARATIVE ROLE OF WHOLE BODY X-RAY (WBXR), 18F-FLUORODEOXY GLUCOSE POSITRON EMISSION TOMOGRAPHY (FDG-PET) AND MAGNETIC RESONANCE IMAGING (MRI) IN EARLY DETECTION OF BONE DISEASE IN MYELOMA PATIENTS
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WBXR is the standard method for the diagnosis of bone disease in Multiple Myeloma (MM). Recently in some selected case has been stressed the importance of PET or MRI. From the beginning of 2005 we started a trial to compare the accuracy of FDG-PET versus the golden standard method in MM staging. The endpoints of the study were to evaluate the efficacy of the three radiologic methods in early detection of bone disease and the impact on treatment program. We evaluated 13 patients with MM1A or MGUS, 14 patients in CR after autografting and 4 patients with uncertain staging. Fourteen patients were previously treated with autografting and 17 patients had no prior therapy. The M-component isotope was IgGk: 10, IgGlamba: 9, IgAκ:3, IgAλ: 5, microk: 1, microλ: 1 and 3 were non secretory. Median age was 61 years (58-81); the bone marrow PC was 0-80% (mean 10%), the β2M ranged 1.1-3.4 mL% (mean 2.3 mL%), the Hb level ranged 8.2-15.7 (mean 14) and the MC component level 0-8425 mg% (mean 1922 mg%). Six patients had 13 q- abnormalities. FDG-PET was performed within 6 weeks of WBXR and MRI. Whole-body (including skull, upper limbs and femora) was carried out using standard procedures. MRI was performed according with the standard method including the skull, the spine and femora. WBXR was positive in 1/13 patients. PET was negative in the entire group and in 2 cases MRI was positive. RX was concordant in 1patient. Of the 14 patients responding after autografting, 3 were in PR, 5 in CR and 6 in relapse. Of 5 remitters patients with WBXR, 3 were PET negative and 2 PET positive; moreover, 1 was MRI positive and 4 MRI negative. All the 3 PR patients resulted positive to WBXR but 2 of them resulted MRI and PET negative. Of the 6 patients in relapse, 2 were positive to WBXR, 4 positive with FDG-PET and 6 with MRI. The 4 patients with uncertain staging resulted WBXR negative; 2/4 were PET positive and 3/4 MRI positive. In conclusion, our preliminary data seems to demonstrated that MRI is the most effective method to detect bone disease in patients with early MM or in MM relapsing after autografting.

PO-255
BORTEZOMIB, LIPOSOMAL DOXORUBICIN AND DEXAMETHASONE COMBINATION THERAPY FOR PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA
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The combination of Bortezomib, liposomal Doxorubicin and dexamethasone (PAD) had proven effective in phase II clinical trials for untreated and previously treated patients with multiple myeloma. Liposomal doxorubicin has a lower cardiac toxicity and comparable efficacy than standard doxorubicin. It can be safely used in elderly patients or in those with prior exposure to anthracyclines. Finally, Bortezomib and liposomal doxorubicin have shown, in vitro, enhanced antitumor efficacy. In this report we present the preliminary results of the use of PAD combination in relapsed-refractory patients with multiple myeloma. Twenty-six patients with refractory or relapsed multiple myeloma, median age 61 (range 45-76) were enrolled since November 2005. 21 among them were evaluable as they have received at least three cycles of chemotherapy. Bortezomib 1.3 mg/mq iv days 1,4,8,11 was administered, liposomal doxorubicin 30 mg/mq on day 1 and dexamethasone 40 mg on days 1-4. This treatment has been repeated every 4 weeks until the 6 cycles. Among these 21 evaluable patients: 10 present a clinical stage IEA, 6 IIIA, 4 IIIB and 1 IIB. Median time from diagnosis was 38.5 months (range 9-121). Of them 4 were primary refractory patients and 7 second line treated survivors. Th first therapy lines were a median of 2 (range 2-5). Six received autotransplant with Melphalan 100 or 200 mg/mq as conditioning regimen, 2 alloBMT, 17 Thalidomide plus dexamethasone and 19 chemotherapy cycles containing anthracyclines. At present 8 patients have completed the treatment, whereas the all the others are being treated and two of them have received 5 cycles, five 4 cycles, two 3 cycles. Haematological toxicity has grade 2 of neutropenia for two patient and grade 2 thrombocytopenia for two. One patient had shown grade 3 neurological toxicity, two grade 3 gastroenterological toxicity (diaree) and two grade 3 fatigue. Two patients had HZ infection. Other grade 2 adverse event were: nausea (1), FUO (4), sensitive polineuropathy (3), fatigue (1). For one patient we have performed a dose reduc-
who have completed 6 cycles of the treatment were alive in PR at +12-4 months from the end of the therapy. Among non-responsive patients: 4 died +3, +5, +6, +7 months. The PAD regimen has demonstrated an acceptable toxicity, no evidence of cardiac toxicity. The previous use of anthracyclines does not seem influence the response in this setting of patients. Although an adequate follow-up is needed to draw any conclusions, this regimen appears very effective and we obtained a reduction of monoclonal component > 50% for 51% of the refractory and relapsed patients with multiple myeloma.

**PO-256**

**Bortezomib and Dexamethasone Administration in Multiple Myeloma after Autologous Stem Cell Transplantation.**

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Recent progress in the treatment of multiple myeloma (MM) has significantly changed the outcome for myeloma patients. Consolidation with high-dose therapy followed by single or double autologous stem cell transplantation has improved response and survival of patients with MM; however, due to its high relapse rate the disease remains incurable. The emergence of novel agents offer potentially significant advances in the treatment of MM. Bortezomib, a selective proteasome inhibitor, has proven to be safe and effective in patients with relapsed and/or refractory MM as monotherapy in phase II/III clinical trials and has produced promising activity given in combination with cytotoxic agents and/or dexamethasone. In our institution 10 newly diagnosed patients (4 F and 6 M, median age 63 years, 6 of 10 patients with stage II/III MM) received 4 courses of VAD (vincristine, doxorubicin, dexamethasone) and high-dose melphalan (200 mg/m²) with subsequent autologous peripheral blood stem cell transplantation (PBSCT). At one month from the treatment all the 10 patients had a partial or complete remission of disease according to EBMT criteria: four complete (negative immunofixation) and six partial (reduction of M-component > 50-75%) remissions were achieved. We decided to treat all patients with bortezomib and dexamethasone as maintenance therapy. All the 10 patients had a partial or complete remission of disease. At the present, (month +24) all patients are alive and 8 out of 10 patients are still in CR. Our results seem to suggest a role for bortezomib, together with dexamethasone, in consolidation/maintenance therapy after PBSCT in MM. This therapy shows promising activity given in combination with cytotoxic agents and/or dexamethasone as monotherapy in phase II/III clinical trials and has produced promising activity given in combination with cytotoxic agents and/or dexamethasone.

**PO-257**

**Bortezomib, Doxorubicin and Dexamethasone (PAD) for Untreated Patient with Aggressive Multiple Myeloma: A Case Report**


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Multiple Myeloma (MM) patients with unfavourable cytogenetics such as 13q deletion [del(13)q], have a poor prognosis irrespective of treatment with conventional chemotherapy or autologous stem-cell transplantation. In patients with MM, Bortezomib is active even in the presence of adverse prognostic factors and it is now considered an effective therapy. Nevertheless, the use of bortezomib-based regimens as front line therapy remains under investigation. Case report. In September 2006, a 59 year old male was referred to our Haematology Unit with a mild anaemia (Hb 12.5 gr/dl), hyperproteinaemia (10.5 gr/dl) with hypergammaglobulinemia (49.8%) and an IgG lambda monoclonal component of 4.9 gr/dl. Creatinine was elevated (10.5 mg/dl) and renal function was normal. A bone marrow biopsy revealed the presence of plasma cells for 80% of the cellularity. Conventional cytogenetics on bone marrow metaphases showed a normal karyotype, but fluorescence in situ hybridization analysis (FISH) was positive for del13q34. Skeletal surveys revealed multiple osteolytic bone lesions. A diagnosis of IgG lambda multiple myeloma, stage IIIA (Salmon and Duree), with adverse prognostic factors was made. Two weeks later the disease was complicated by two pathological fractures of the 9th and 11th ribs. The patient was treated initially with involved field radiotherapy and thalidomide plus dexamethasone and subsequently with PAD regimen consisting of Bortezomib, Dexametason and Doxorubicine, for a total of four cycles. A very good partial remission was obtained. Monoclonal component dropped to 0.58 gr/dl and a bone marrow biopsy revealed only 5-10% of bone marrow plasmacytomas. Because of the absence of an HLA-identical sibling donor, the patient will be then considered for a program of high-dose therapy followed by a tandem transplantation (autologous and reduced intensity conditioning (RIC) allo-transplantation from an unrelated donor).

**PO-258**

**Multiple Myeloma: Discordant Response between Medullary and Extramedullary Disease after Thalidomide Based Regimens. Report of Three Cases**

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Thalidomide-based regimens (TBR) are now used in first line treatment of Multiple Myeloma (MM) and in refractory or relapsed disease. There are some reports in literature about discordant responses between serum monoclonal protein levels and (plasma cell infiltrate in the bone marrow) and the extramedullary disease. We report here three cases of MM who were treated at our Department with TBR as a first line therapy. These patients experienced extramedullary progression during TBR despite a bone marrow and laboratory response. The features of cases are reported in the following Table 1.

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Myeloma type (stage)</th>
<th>Year of diagnosis</th>
<th>Baseline M-protein (g/l)</th>
<th>Months to start TBR</th>
<th>Type of extramedullary progression</th>
<th>Appearance of extramedullary mass</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>63</td>
<td>IgG/K/3</td>
<td>2006</td>
<td>40</td>
<td>2</td>
<td>M-protein at extramedullary progression (g/l)</td>
<td>Appearance of extramedullary mass</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>54</td>
<td>IgK/L</td>
<td>2006</td>
<td>41</td>
<td>3</td>
<td>M-protein at extramedullary progression (g/l)</td>
<td>Appearance of extramedullary mass</td>
<td>Death</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>55</td>
<td>IgG/K/3 + paravertebral mass</td>
<td>2006</td>
<td>21</td>
<td>4</td>
<td>M-protein at extramedullary progression (g/l)</td>
<td>Appearance of extramedullary mass</td>
<td>Death</td>
</tr>
</tbody>
</table>

These findings confirm that some pts under thalidomide based therapy may progress with extramedullary involvement despite a systemic response with bone marrow plasma cells (BMPC) and M-component reduction. The mechanism to explain this discordance is not yet clarified but some data suggest a possible dedifferentiation of MM plasma cells during thalidomide therapy with acquisition of a new malignant phenotype allowing to escape the drug effect.

References:

PO-259
ZOLEDRONIC ACID INCREASES HIGH-DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C) AND DECREASES LOW-DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C) IN MULTIPLE MYELOMA PATIENTS

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Background. Zoledronic acid (ZA) represents a novel amino bisphosphonate (N-BP) in the prevention and treatment of bone metastases that has also anti-tumor activity. Several potential mechanisms have been proposed: direct cytotoxic or cytostatic effects on tumor cells, inhibition of tumor cell invasion of the bone and anti-angiogenic effects. Even though the mechanisms of action are not completely known, it has been demonstrated that N-BP could induce apoptosis of macrophages and osteoclasts by inhibiting the mevalonate pathway. By inhibiting farnesylpyrophosphate synthetase activity, N-BP could interfere with cholesterol synthesis, which represents a final product of the mevalonate–squalene pathway. Recently, a remarkable effect on lipid metabolism was reported in osteoporotic women with a single infusion of neridronate, a potent amino bisphosphate. The effect of N-BP on cholesterol in cancer patients, and particularly in multiple myeloma (MM) patients, has never been reported; moreover ZA has shown superior efficacy in vitro and in vivo on MM cells compared to previously used BP. Patients and Methods. Based on this background, 26 smoldering MM patients (SMM) were studied at our institution to investigate the effect of ZA on lipid metabolism.

PO-260
BORTEZOMIB IN RELAPSED/REFRACTORY MYELOMA PATIENTS: A SINGLE-CENTER EXPERIENCE

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Introduction. We analyzed the efficacy, safety, and cost effective of Bortezomib in refractory or relapsed MM patients. Methods. We reviewed 19 patients with relapsed or refractory multiple myeloma treated with Bortezomib at our institution between October 2005 and March 2007. 8/19 pts (42%) received Bortezomib as second line of therapy, post stem cell transplant (group A) and 11/19 (58%) received more than 2 lines of prior therapy (group B). Patients received Bortezomib 1.3 mg/m2 as an intravenous bolus twice a week for 2 weeks, on days 1, 4, 8 and 11, in a 21 day cycle with addition of Dexamethasone 20 mg on the day of and the day after Bortezomib for up to 4 cycles. PFS, TTP and DOR were evaluated. Results. The median age was 64 yrs (42-75). Only 8 pts (42%) completed the study efficacy at the same time.

Conclusions. ZA has a consistent effect on lipid metabolism. Further studies are needed to confirm these findings.

Figure 1. Percent changes of Total cholesterol (TC) (A) and LDL cholesterol (LDL) (B) in treated and untreated patients (the values are expressed as mean±SE). * p<0.05 treated (ZA) vs no treated (NO ZA) patients.

In particular the present study aimed to evaluate cholesterol and lipoprotein serum levels in patients with MM treated with intravenous zoledronate (ZA). Twenty-six untreated consecutive patients (14 males/12 females; median age 71.4±7.8, range 49-82 years) with SMM (14 IgG/k, 2 IgG/λ, 8 IgA/k and 2 IgA/λ) were recruited between October 2004 and March 2005 our institution. In order to enter the study, patients had to be not on treatment for hypercholesterolemia. After baseline evaluation, SMM patients were sequentially assigned to receive no treatment (10 patients) or ZA (16 patients), 4 mg administered as a 15 min i.v. infusion at baseline and after 1, 2, 4 and 6 months. In all subjects total cholesterol (TC), tryglycerides (TG), and high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) were measured before infusions (ZA) at baseline and at 3-month intervals up to 6 months. Serum and uranary calcium, serum phosphorus and serum creatinine were analyzed with standard methods. Bone alkaline phosphatase (Bone ALP) were measured as markers of bone remodelling using ELISA as described elsewhere. Data distribution was expressed as mean±SD. Two-Sample T-test and Chi-square test were independent data were used to test the differences between groups. Analysis were performed using SPSS software for Windows, version 10.0 (SPSS ltd., Chicago, IL, USA). Results. All patients completed the study. They did not modify their diet, and were not given other drugs that were able to interfere with lipid and bone metabolism. The physical activity and mean body weight did not change during the study period. In the control group (not treated) the biochemical profile did not change during study period. Mean serum TC and LDL-C early significantly and progressively decreased in the treated patients. In fact, after 1 month TC decreased by 8%, after 3 months was decreased by 10%, (p=0.003) and was decreased by 13% at 6 months (p=0.00). In the same way, LDL-C was also reduced by 12%, 15%, and 21% at 1, 3, and 6 months of treatment (p=0.001; p=0.001; p=0.006) (Figure 1). Interestingly, analysis of results revealed an early and significant reduction of serum CTX in treated patients, and the positive effect of ZA on bone tissue was confirmed by the improvement in lumbar spine BMD at 6 months (+8.8%±2.6 vs baseline; p<0.05). These findings were also accompanied by an early and significant reduction of bone telopeptides of Type-I collagen (CTX) in treated patients, meaning treatment efficacy at the same time.

Conclusions. ZA has a consistent effect on lipid metabolism. Further studies are needed to confirm these findings.
showed a moderate activity, mostly PR. The DOR is similar to the one reported by conventional salvage chemotherapy; however the latter is less expensive and toxic. We believe that Bortezomib is not cost effective agent in relapsed, elderly MM patients. Moreover we believe more studies are needed to warrant the role of Bortezomib in a younger subset of patients with earlier disease.

**PO-261**

**BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA: A SINGLE-CENTRE RETROSPECTIVE STUDY ON 45 PATIENTS**


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Forty-five relapsed/refractory multiple myeloma (MM) patients (pts) were treated with Bortezomib (Btz). Pts’ characteristics were: 27 M/18 F; median age 55 years (range:36-65), median number of prior regimens 2 (range:1-6), prior high-dose therapy in 29 pts (64%) and thalidomide in 34 (76%). Btz 1.3 mg/m² was given on days 1,4,8,11 every 21 days. Eleven pts were treated with Btz alone, 34 with Btz and Dexamethasone (Dex) 20 mg on days 1,2,4,5,8,9,11,12 every 3 weeks. Five out of 45 pts were retreated with Btz in combination with Dex (6 pts) or Melphalan 9 mg/m² on days 1-4 (1 pt). Grade 3/4 neutropenia (grades 1-4 evoked) was observed (VMP) (2 pts). Forty pts were evaluable for response and toxicity. The median number of cycles delivered was 5 (range:2-8). Twenty-one pts (52.5%) achieved at least a partial response, including 2 (5%) complete remissions. Two out of 5 pts retreated with Btz obtained a clinical response. The median time to the best response was 42 days (range:21-225). After a median follow-up of 10.8 months (range:1.5-29.8), 37 pts (82%) showed a response, 21 (46.6%) achieved a CR, 2 (11%) a very good partial remission (VGPR) (more than a partial remission (PR)] was 61% (11 pts). Eight patients (17%) discontinued treatment because of PN. Complete resolution or improvement of PN was observed in the majority of pts after a median time of 128 days (range:66-340). The occurrence of PN was not influ- enced by age, sex, number of prior therapies or prior use of thalido- mine. Twelve pts (30%) had gastrointestinal symptoms, of mild or mod- erate intensity in most cases. However, grade 3 diarrhea led to Btz discontinuation in 3 pts (7.5%). Grade 2/3 thrombocytopenia was observed in 13 pts (32.5%) and grade 3/4 neutropenia in 2 (5%). Herpes zoster occurred in 6 pts, but this complication was no further observed after the introduction of antiviral prophylaxis. In conclusion, the analysis of this single centre experience shows that Btz is effective in relapsed/refractory MM producing good responses even in heavily pre- treated pts, but the duration of response is short. Side effects, which are usually manageable and reversible, PN is the most frequent and clinically relevant. Retreatment with Btz-based therapies in pts previously responsive to Btz is effective in less than a half of pts.

**PO-262**

**INEFFICACY OF BORTEZOMIB ON PLASMA CELL LEUKEMIA (PCL): SINGLE INSTITUTION EXPERIENCE**

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**Introduction.** Recent international reports indicate that Bortezomib is highly efficient in the treatment of PCL, a rare form of plasma cell dyscrasia characterized by very poor prognosis despite conventional aggressive chemotherapy including autologous and allogeneic transplantation. We are describing three clinical cases, 2 primary PCL and 1 secondary PCL, treated with Bortezomib alone or in combination with other drugs. **Patients and Methods.** Patient 1. In March 2006 a 64-year-old woman was diagnosed with primary PCL. The patient was refractory to Talidomide (at dosage of 200 mg/d) + Dexamethasone and subsequently Cyclophosphamide + Dexamethasone. In November 2006 progression into PCL was observed; the patient developed severe anaemia (Hb 6.9 g/dL), leukocytosis (WBC 15.0×10⁹/L with 33% of circulating plasma cells) and thrombocytopenia (PLT count <20×10⁹/L). Therapy with Bortezomib 1.3 mg/m² on days 1, 4, 8, 11 associated to Dexamethasone 20 mg i.v. on days 1–4, 8–11 and oral Cyclophosphamide 50 mg daily on days 1–21 was started. The treatment failed and the patient died some days later for acute renal failure. Patient 2. In July 2004 a 71-year-old man was diagnosed with primary PCL. The patient was refractory to VAD-like protocol. A further treatment with Cyclophosphamide + Dexamethasone induced a decrease of the peripheral plasma cell count to 4%, while there was no decrease of the bone marrow infiltration. Therefore, treatment with Bortezomib 1.3 mg/m² on days 1, 4, 8, 11 + Dexamethasone 20 mg i.v. on days 1–4, 8–11 and Oral Cyclophosphamide 50 mg daily on days 1–11 was started as a final attempt. During the fourth cycle the patient developed deterio- ration in his clinical conditions for progression disease and died some months later. Patient 3. In November 2006 a 42-year-old woman was diagnosed with primary PCL. The patient was initially refractory to VAD-like protocol. After two cycles of PAD regimen (Bortezomib 1.3 mg/m² on days 1, 4, 8, 11, Adriamycin 9 mg/m² on days 1-4; Dexamethasone 40 mg on days 1–4, 8–11, 15–18 during cycle 1 and 1→ on subsequent cycles) peripheral blood smear still showed circulating plasma cells. So, the decision for therapy intensification was made and CODOX-M regimen was started; after 1 course, finally, the circulating plasma cells disappeared completely but the serum monoclonal protein levels remained constantly elevated (M-spike 5 gr/dl). Conclusion In view of the lower incidence of this monoclonal gammopathy, there are relatively few reports describing the efficacy of bortezomib in the treat- ment of primary or secondary PCL after multiple salvage therapy. Curr- rently, our clinical data strongly contrast with favourable responses reported by these single experiences; in fact, here we report three clinical cases in which circulating plasma cells persist despite bortezomib therapy. Since it is difficult to identify the prognostic factors foretelling treatment response at this time, data from other groups with larger series of patients are necessary to express a correct judgment about efficacy of bortezomib on PCL.

**PO-263**

**TOLERABILITY AND EFFICACY OF BORTEZOMIB CONTAINING REGIMENS FOR THE TREATMENT OF ELDERLY PATIENTS AFFECTED BY RELAPSED/REFRACTORY MULTIPLE MYELOMA**


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**Background.** Bortezomib (Velcade®, V) has become the standard care for relapsed/refractory multiple myeloma (MM) patients. Moreover, this group of patients achieved a higher overall (ORR) and complete response (CR) rate when V was administered in association with other drugs such as Dexamethasone (D), pegylated liposomal doxorubicin (PLD), mel- phalan (M) and thalidomide (T). **Aims.** The purpose of this study was to assess the tolerability and efficacy of V containing regimens in elderly patients affected by relapsed/refractory MM. **Patients.** Between November 2005 and April 2007, 18 patients affected by relapsed/refractory MM were treated with V containing regimens. This sample had the following demographics: median age of 71 years (range 58-81 years) isotypes IgG 50%, IgA 22%, light chain 28%, 39% were female. The median number of prior therapy was 1 (range 1-3), 50% were refractory to prior chemotherapy. The regimen of chemotherapy administered was: PAD (V + PLD + D) in 6 patients, VTD (V + T + D) in 4, VT (V + T) in 1, VD (V + D) in 5, MPTV (M + prednisone + T + V) in 2. Results. All patients received 1.3 mg/m² of V for 4 doses per cycle; in the PAD, VTD and VD regimen V was administered on days 1, 4, 8 and 11 every 3 weeks, while in the MPTV on days 1, 8, 15 and 22 every 5 weeks. The median number of cycles administered was 4 (range 3-9). All patients received prophylactic anti-virals. Notable hematologic AEs were record- ed in 77% of patients (14 pts) and included neutropenia (grade 3 in 5% of pts) and thrombocytopenia (grade 3 in 72%), no one episode of febrile neutropenia was observed. Non-hematologic WHO grade 3 were seen in 10 pts (55%), while none of the pts showed WHO grade 4 non- hematologic toxicities or VZV reactivation. The most common notable side effects were: fatigue (WHO grade 3 in 25%), sensory motor neuropathy (WHO grade 3 in 12%), diarrhea (WHO grade 3 in 28%), V dose has been reduced in 10 (55%) out of 18 pts to 1.0 mg/m² because of the persistence of WHO grade 3 non-hematologic toxicities. The ORR (more than a partial remission (PR)) was 61% (11 pts). Eight patients (44%) achieved a CR, 2 (11%) a very good partial remission (VGPR).
and 1 (6%) a PR. Conclusions. These preliminary data suggest that V containing regimens demonstrated substantial clinical activity and should be considered an appropriate treatment even in elderly patients with relapsed/refractory MM. However, V should be administered at a lower dosage (i.e. 1 mg/m²) to avoid the high incidence of non-hematologic toxicity.

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BORTEZOMIB IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS. EXPERIENCE OF A SINGLE CENTER
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Introduction. We report the results of a 3-year experience using Bortezomib, a proteasome inhibitor, approved for relapsed or refractory Multiple Myeloma (MM). Methods. Between April 2003 and December 2006 we used Bortezomib to treat 33 MM pts, 20 males and 13 females, with a median age of 61.5 years (range 32-76); 22 (66.7%) pts were IgG, 9 IgA (27.3%) and 2 (6%) light chain MM. The median observation time from diagnosis to Bortezomib therapy was 53.3 months (range 11-151). According to the Durie and Salmon's classification system, all pts were in stage III and had been previously pretreated with a median of two lines of therapy (range 1-5), including alkylant agents, stem cell transplantation and Thalidomide, resulting refractory (8 pts) or in relapse (25 pts) after the last treatment. Bortezomib was administrated at 1.3 mg/m² on days 1, 4, 8 and 11 with a 10 day rest period, constituting a 21-day cycle for 8 cycles. The median number of cycles administrated was 6 (range 2-8); considering as evaluable pts treated with at least 3 cycles of therapy, we included 31 pts: 19 completed the whole treatment, while 12 had to discontinue (1 because of an allogenic transplantation, 6 due to adverse events and 5 because of progressive disease). Results. Our primary endpoint was to obtain a reduction in the monoclonal component (MC) of at least 25%. According to the standard Blàde criteria, 18 pts (58%) proved responsive to treatment: 1 (5.5%) obtained a complete response with negative immunofixation, 14 (77.8%) achieved a partial response with a reduction of the MC greater than 50% and 3 (16.7%) showed a minimal response. The median response duration was 6 months (range 1-28), with 4 pts (22.2%) still responsive after a median observation time of 13.5 months. Among the other responding pts, 10 (55.6%) relapsed and were treated with different lines of therapy, 4 (22.2%) died because of disease progression. 13 pts (42%) resulted resistant to Bortezomib: 6 because they reached a decrease of MC smaller than 25% and 7 due to progressive disease. Among these latter pts, 8 underwent other treatment modalities and 5 died. Major adverse events included thrombocytopenia, gastrointestinal symptoms and peripheral neuropathy. Discussion. Considering MM currently incurable, these results confirm the efficacy of Bortezomib in second-line and beyond MM pts. Nevertheless, its toxicity and the reversibility of its actions must be taken into account in designing future clinical trials.

PO-265
DETECTION OF CIRCULANT MONOCLONAL PLASMA CELL IN THE PERIPHERAL BLOOD OF PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA AND MGUS
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The aim of this study was to quantify the number of circulant monoclonal plasmacells in the peripheral blood of patients with newly diagnosed multiple myeloma (MM) and in the monitoring of these patients and in patients with monoclonal gammapathies of uncertain significance (MGUS). We think that circulating plasma cells (PCs) can be detected in the peripheral blood of a great proportion of patients with MM and with MGUS. The appearance of circulating PCs in the blood may be simply a reflection of a tumor mass, but it could also represent differences in the microenvironment and, in other words, signifies more aggressive disease. To these purposes we analyzed by flow cytometry peripheral blood samples from 10 patients without hematological disease, and from 15 patients with a diagnosis of de novo MM and from 20 patients with MGUS diagnosed on standard criteria, which were referred to our Division from march 2004 to october 2006. Flow cytometry analysis was conducted with lyse no wash method. Cells were stained with fluorescence-labelled CD38 FITC, CD56 PE, CD19 PE-Cy5, CD138APC and CD45 PB antibodies (from Dako, Glostrup, Denmark). Circulating PCs were detected in all MM patients with the median PC count of 7 per 1.000.000 events. Circulating PCs were found in all patients; 5 MM patients had 1 to 10 circulating PCs. The remaining 10 patients had more than 10 circulating PCs. Circulating PCs were also detected in MGUS patients with the median PC count of 3 per 1.000.000 events. We didn't found any correlation between the degree of narrow involvement by PCs and the number of circulating PCs. Our preliminary results show that PCs in peripheral blood represent differences in myeloma disease biology.

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BORTEZOMIB IN ASSOCIATION THERAPY IN RELAPSED, REFRACTORY MULTIPLE MYELOMA: EXPERIENCE OF A SINGLE CENTER
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Background. Bortezomib is a proteasome inhibitor inducing apoptosis of tumor cells in vitro. Results of APEX and SUMMIT trials showed the safety and efficacy of bortezomib in refractory or relapsed Multiple Myeloma (MM) patients. Aims. To study the activity of bortezomib in combination with i.v. dexametasone (B-Dexa) or oral melphalan/prednisone (BMP), or i.v. cyclophosphamide/dexametasone (BCD) we analyzed 20 relapsed/refractory outpatients MM. Methods. 20 patients having undergone > 1 prior lines of therapy were treated: 12 with B-Dexa, 4 with BMP, and 4 with BCD. The clinical features of 20 patients were: 13 males and 7 females, with a median age of 65 years (range 52-78 years), immunoglobuline type IgG in 13 and IgA in 7. Seven patients had already received one treatment line and 13 had been previously treated at least with two chemotherapy lines. B-Dexa consisted of i.v. Bortezomib (1.3 mg/m² on days 1,4,8,11) plus dexamethasone (20 mg) in the same days for 8 cycles each 21 days. BMP consisted of i.v. Bortezomib (1.3 mg/m² on days 1,4,8,11,22,25,29,32) plus oral melphalan 6 mg/m² and oral prednisone 60 mg/m² on days 1-5, for 4 cycles each 40 days. BCD treatment consisted of i.v. Bortezomib (1.3 mg/m² on days 1,4,8,11,22,25,29,32) plus oral cyclophosphamide 150 mg/m² on days 1,8,15,22 for 4 cycles each 40 days. B-Dexa, BMP and BCD were followed by 3 consolidation treatment with Bortezomib (1.3 mg/m² on days 1,8,15,22) if patients reached partial or complete remission. Results. 10 patient achieved a partial remission (50%), 5 complete remission (25%), 2 minimal response (10%), 3 (15%) were refractory to treatment. In our experience in each group of treatment (B-Dexa, BMP, BCD) we observed significant differences in the number of responses (B-Dexa 58%, BMP 38%, BCD 25%) between patients with relapsed/refractory MM and in patients with a diagnosis of de novo MM. Conclusions. Initial results in our experience showed that combination of bortezomib with dexamethasone or melphalan/prednisone or cyclophosphamide/dexamethasone is a promising well tolerated association for advanced MM.

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EVOLUTION OF EMPERIC ANTIFUNGAL THERAPY IN A BONE MARROW TRANSPLANTATION UNIT

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Introduction. The role and significance of empirical antifungal therapy in the setting of febrile neutropenia (FN) is still a matter of debate, particularly in the field of myeloablative regimens and hematopoietic stem cell transplantation (HSCT). Myeloma 6, lymphoma 8). The median age was 51 years (range 21-63); 18/30 pts underwent SCT from unrelated and 12/30 from related donors; 17/30 (57%) pts received a reduced intensity conditioning regimen (RIC). Antiviral prophylaxis consisted in acyclovir in all cases. At the onset of CMV infection 21/30 (70%) pts had an acute or chronic graft versus host disease for which were received therapy including prednisone and/or other immunosuppressive agents. The CMV antigenemia assay was positive in all cases with a mean number of positive nuclei of 22±43. The first 15 pts were treated with VGC 900 mg twice a day (GROUP A) and the following 15 pts with VGC 450 mg twice a day (GROUP B); the treatment was continued in all cases until the CMV antigenemia became negative in two consecutive samples or until the treatment failure (increase of antigenemia in two consecutive samples). The two groups resulted well balanced. Results. Overall 27/30 cases obtained a clearance of antigenemia (14/15 in the GROUP A and 13/15 in the GROUP B). The median duration of VGC therapy was 18 (range 8-64) and 22 (range 8-61) days, respectively. No cases of CMV disease were reported. Fifteen (50%) pts experienced one or more recurrence of CMV infection without significant differences in the two groups (8/15 vs 7/15) and seven cases required intravenous therapy with foscavir. Only three patient (2/3 in the group A and 1/3 in the group B) developed a mild deterioration of renal function that required dose adjustment (VGC 450 mg/day in 1/3 and VGC 900 mg/day in 2/3). None of the pts developed gastrointestinal disorders grade II WHO or more; mild and transient neutropenia was reported in 12/30 (40%) cases, transient anemia in 9/30 (30%) and transient thrombocytopenia in 12/30 (40%) pts without significant differences between two groups. Conclusions. 1) Preemptive therapy with VGC after related and unrelated allogeneic SCT seems to be safe and effective with a rapid clearance of antigenemia. 2) The optimal dose and duration of VGC therapy, in this setting, need to}

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PARVOVIRUSES PARV4/5 IN BLOOD DONORS AND TRANSPLANT PATIENTS FROM ITALY

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We performed a retrospective molecular study by polymerase chain reaction (PCR) for the presence of the novel parvoviruses, PARV4/5, in four groups of 417 Italian HIV negative cases. We found 3 positive cases, including 2 out of 126 renal transplant recipients and 1 out of 84 patients with a suspected viral disease, while 0 of 100 blood donors from Northern Italy were positive, on single round PCR. One blood donor sample and the positive rate did not increase in the other groups, on nested PCR. In the first two groups, PARV4/5 was detected only in the sera but not in the peripheral blood mononuclear cells (PBMCs), suggesting that PBMCs are not a major site of viral replication. None of the 107 allogeneic bone marrow (BM)/peripheral blood stem cell (PBSC) transplant recipients was PARV4/5 positive. The detection of PARV4/5 in the serum collected at 12 months after transplantation, was not associated with the occurrence of any symptoms, in the 2 renal recipients. Both recipients were, however, negative before, 6, and 24 months after transplantation. This is reminiscent of B19 infection in solid organ transplant recipients, in whom B19 infection rate is low (1, 4%-1.8%) and asymptomatic, in most of the cases. PARV4/5 was detected in the serum from 1 patient with Wegener granulomatosis, under long-term treatment with steroids, concomitant with the development of a clinical syndrome for which a viral aetiology was suspected, including fever, severe anemia, a post-infectious glomerulonephritis, and erythroid hypoplasia, on BM examination, but resulted negative for the most common viruses, including B19. Single-cell PCR performed on the DNA extracted from isolated BM erythroid and myeloid progenitors in the BM tissue biopsies, collected 2 days before death, and at autopsy, resulted PARV4/5 negative. While the PARV4/5 viremia, in the absence of other known viral agents, suggests a possible contribution of this novel parvovirus to the patient’s clinical syndrome, the absence of the virus in the BM cells suggests that its in vivo tropism may markedly differ from that of B19. In conclusion, the frequency of PARV4/5 infection is very low in the general Italian population, while being slightly higher in certain subgroups of iatrogenically immunosuppressed subjects. A possible pathogenic role of PARV4/5 infection needs to be confirmed in further studies.

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VALGANCICLOVIR (VALCYTE) 900 MG/DAY HAS A COMPARABLE EFFICACY THAN VALGANCICLOVIR 1800 MG/DAY AS A PREEMPTIVE THERAPY OF CYTOMEGALOVIRUS DISEASE IN RECIPIENTS OF ALLOGENEIC STEM CELL TRANSPLANT (SCT)

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Introduction. Cytomegalovirus (CMV) infection is a common complication after allogeneic SCT. Valganciclovir hydrochloride (VGC) is a pro-drug of ganciclovir (L-Valyl ester), orally available, that is currently approved for CMV infection in high-risk (donor positive, recipient negative) solid organ transplants (1800 mg/day as a standard dose). The efficacy of VGC for this indication is not well defined. A recent study in allogeneic SCT (PO-269 Italy) has not been established. Patients and Methods. The primary aim of our study was the assessment of efficacy and safety of two dosages of VGC (1800 mg/day and 900 mg/day) as preemptive therapy of CMV disease after allogeneic SCT. During a 12 months period VGC was administered to 30 consecutive outpatient (pts) with a CMV infection which was diagnosed after a median time of 86 days (range 59-480) from transplantation. There were 17 males and 13 females (myelodysplosis 3, leukemia 13, myeloma 6, lymphoma 8). The median age was 51 years (range 21-63); 18/30 pts underwent SCT from unrelated and 12/30 from related donors; 17/30 (57%) pts received a reduced intensity conditioning regimen (RIC). Antiviral prophylaxis consisted in acyclovir in all cases. At the onset of CMV infection 21/30 (70%) pts had an acute or chronic graft versus host disease for which were received therapy including prednisone and/or other immunosuppressive agents. The CMV antigenemia assay was positive in all cases with a mean number of positive nuclei of 22±43. The first 15 pts were treated with VGC 900 mg twice a day (GROUP A) and the following 15 pts with VGC 450 mg twice a day (GROUP B); the treatment was continued in all cases until the CMV antigenemia became negative in two consecutive samples or until the treatment failure (increase of antigenemia in two consecutive samples). The two groups resulted well balanced. Results. Overall 27/30 cases obtained a clearance of antigenemia (14/15 in the GROUP A and 13/15 in the GROUP B). The median duration of VGC therapy was 18 (range 8-64) and 22 (range 8-61) days, respectively. No cases of CMV disease were reported. Fifteen (50%) pts experienced one or more recurrence of CMV infection without significant differences in the two groups (8/15 vs 7/15) and seven cases required intravenous therapy with foscavir. Only three patient (2/3 in the group A and 1/3 in the group B) developed a mild deterioration of renal function that required dose adjustment (VGC 450 mg/day in 1/3 and VGC 900 mg/day in 2/3). None of the pts developed gastrointestinal disorders grade II WHO or more; mild and transient neutropenia was reported in 12/30 (40%) cases, transient anemia in 9/30 (30%) and transient thrombocytopenia in 12/30 (40%) pts without significant differences between two groups. Conclusions. 1) Preemptive therapy with VGC after related and unrelated allogeneic SCT seems to be safe and effective with a rapid clearance of antigenemia. 2) The optimal dose and duration of VGC therapy, in this setting, need to
be established with additional prospective studies but in our experience the low dose of VGC (900 mg/day) has comparable efficacy and safety profile than the higher dose (1800 mg/day) supporting the low dose use.

3) Regular blood counts and creatinine should be performed in all cases (at least once a week) to early detect cytopenia or renal toxicity.

PO-270
SEVERE OPPORTUNISTIC INFECTIONS AFTER RITUXIMAB TREATMENT FOR REFRACTORY OR RELAPSING THROMBOTIC THROMBOCYTOPENIC PURPURA: A CASE REPORT
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Introduction. Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease characterized by thrombocytopenia, microangiopathic hemolytic anemia and predominantly neurological symptoms. TTP is associated with a deficient or dysfunctional von Willebrand factor (VWF)-cleaving metalloprotease ADAMTS13. Acquired disease is usually related to the presence of autoantibodies against the ADAMTS13 enzyme. Malignant disease may cause TTP by ADAMTS13 inhibition; however, persistent disease or relapse can occur. In these patients, second-line treatments including immunosuppressive agents have been used with variable success. Rituximab has been shown to provide sustained remission in patients with refractory or relapsing TTP. Since now, no major side effects have been described in literature following Rituximab use in TTP. Case Report. Here we describe a major infectious complication in a 46 years old man affected by idiopathic TTP, who attended our department in November 2006. No relevant disease in the past. We demonstrated the presence of ADAMTS13 inhibitors with a residual enzyme activity below 1%. He received five courses of TPE with high doses corticosteroids (500 mg for three days and then tapering) obtaining complete remission. Two weeks later disease relapsed. He was treated with four weekly doses of Rituximab (375 mg/mg), obtaining a complete remission. He did not receive any antimicrobial prophylaxis. Before starting Rituximab infusions, the absolute lymphocyte count was 850/mmc (β 2 cells: 2%; CD4:10%;CD8:50%; CD4/CD8: 0.19), while the absolute neutrophil count (ANC) was 250×10^3/mmc. Two weeks after the end of treatment, the patient showed a severe neutropenia (ANC<50/mmc), while absolute lymphocyte count was above 1000/mmc. No hypogammaglobulinemia was observed. He started G-CSF, but he soon developed fever and was hospitalized. After few days, he showed a progressive decrease respiratory exchange (TTP-crisis). Pneumocystis jiroveci (Carinii) pneumonia. While recovering from this infection, a severe gastroenteritis due to CMV and pulmonary aspergillosis occurred. He was moved to the Intensive Care Unit but died after three months from the beginning of Rituximab treatment, while TTP was still in remission. Conclusions. Rituximab is an effective therapy in patients with relapsing or refractory TTP; however infectious risk in non-neoplastic patients have still to be investigated.

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PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY AFTER REDUCED-INTENSITY CONDITIONING UNRELATED ALLOGENIC STEM CELL TRANSPLANTATION: A CASE REPORT
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Progressive multifocal leukoencephalopathy (PML) is a JC polyomavirus (JCV) opportunistic infection, which occurs in immunocompromised patients, and leads to oligodendrocyte demyelination. JCV is widely present in the human population and persists in a latent state in the infected cells. PML incidence has increased sharply over the past decade in the same way as AIDS, in these patients PML evolution strongly related to the CD4 lymphocyte count. JCV reactivations occur in around 10% of bone marrow transplant patients; the usual clinical manifestation is hemorrhagic cystitis, whereas PML has been rarely described. Here we report a case of PML in a 50 years old patient with Hodkgin lymphoma treated with four different regimes of chemotherapy and autologous stem cell transplantation. The patient underwent to unrelated allogeneic stem cell transplantation (SCT), after reduced intensity conditioning consisted of Fludarabine/Melphalan/TBI 200 cGy and MabCampath. The chimerism pattern during the post-transplantation period was always full donor, despite the patient showed a moderate cytopenia with a white blood cell count < 3.0×10^3/L (with very low count of CD4 cell < 150/mm^3). Seven months later, while receiving high dose of corticosteroid and CSA for extensive cutaneous chronic graft versus host disease, the patient developed diplopia and left homonymous hemianopsia, associated with a progressive weakness in the left arm. A cerebral MRI scan showed low signal intensity in sequence T1 and high signal intensity T2 subcortical diffuse white matter lesions with no mass effect or mass enhancement. Cerebrospinal fluid cytology, sugar and protein levels were normal. Molecular biology based analysis for Mycobacterium tuberculosis and Cryptococcus neoformans were negative. Real time PCR analysis detected significant levels of JCV DNA in the cerebrospinal fluid (CSF). No CMV, EBV, HHV6, HHV8, HSV1, HSV2, enterovirus genome was found. This finding leads to the diagnosis of PML. However, the patient developed a pancreatic cancer as secondary malignancy with progressive and very rapid liver failure and died 3 months after the start of the neurological symptoms. No antiviral treatment could be undertaken. According to our knowledge this is the first report of PML after a reduced intensity conditioning unrelated allogeneic SCT. Our data support the important role of immunosuppression in the JCV reactivation.

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HHV6 AND MUCOSITIS IN HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Introduction. About 95% of adults have anti-human herpes virus 6 (HHV-6) antibodies. Although HHV-6 is known to reactivate during haematopoietic stem cell transplantation (HSCT), the clinical significance of this phenomenon is controversial. Mucositis represent a devastating and debilitating complication of HSCT that have significantly clinical and economic consequences. Recently we have observed that a group of patients, after complete engraftment, continued to manifest, pain of the mouth, persistent salivation associated to nausea and vomiting with impossibility to resume the solid and liquids foods intake. Moreover, also the white cells and the platelet count showed a progressive decrease. Aim. This observation has suggested to investigate a possible causal relationship between HHV-6 replication and mucositis complications. Patients and method. We used a quantitative PCR test for HHV-6 to assay plasma samples prospectively collected from a cohort of 17 autologous and 21 allogeneic HSCT recipients, 36 of them had suffered by mucositis. In a subset of 29, prevalent mucositis score was 3 or 4. The acute leukaemias and the lymphoproliferative disorders were the major-
PO-273
RETROSPECTIVE EVALUATION OF 314 CENTRAL VENOUS CATHERERS (CVC) IN ADULT HAEMATOLOGY PATIENTS.
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Introduction. Central venous catheter (CVC) infections and thrombosises are the most important complications, that can induce an untimely removal in haematology patients. We analysed the fate of CVCs implanted in our patients, with particular attention to unfavourable events and factors that can predict untimely removals. Methods. We retrospectively analyzed 314 CVCs implanted between January 2002 and December 2005. All patients underwent high dose chemotherapy and/or stem cell transplantation and prophylaxis with ciprofloxacin or levofloxacin during aplasia. A protocol of periodic CVC surveillance cultures was followed. Results. Distribution of patients for age was: 54 (17%) < 40 y; 84 (27%) from 40-60 y; 176 (56%) > 60 y. Diagnosis distribution was: acute leukemia 150 (48%); lymphoma 107 (34%); multiple myeloma 41 (15%); others 16 (5%). 253 (81%) CVCs were Groshong type. CVC insertion was subclavicular in 277 cases (88%) and jugular in 37 (12%). The median CVC duration was 6 months (range 7 days-22 months). Removals were independent of any CVC-related complications in 246 cases (78%), while they were performed in advance as a consequence of infections, malfunction and thrombosis in 39 (12%), 27 (9%) and 2 (1%) cases respectively. At least one episode of subcutaneous phlogosis was evident in 181 cases (57%) (incidence=6.25/1000 CVC-days). 147 subcutaneous infections (81%) were managed without CVC removal. One or more positive surveillance cultures were evident in 79/231 CVSs (34%). A vein thrombosis was demonstrated in 13 (4%) patients. Untimely removals were independent of age, diagnosis, subcutaneous infections and positive surveillance cultures. Positive surveillance cultures were more frequent for Gram (+) than for Gram- bacteria (14). Among 107 positive haemocultures, performed in course of fever, 78 (73%) evidenced Gram+ micro-organisms, 19 (19%) Gram-, 2 (2%) mycetes and 6 (6%) the association of both Gram+ and Gram- in two subsequent cultures. Surveillance and in course of fever cultures were concordant in 83% of cases. Septic events were predicted by both a positive surveillance culture (63% vs 25%, p<0.01) and a subcutaneous infections (43% vs 23%, p<0.01). Conclusions. Untimely CVC removal was present in 22% of patients, mainly for infections. Both positive surveillance cultures and subcutaneous phlogosises are predictive of subsequent septic events and can be useful to choose an empiric antibiotic therapy in case of fever.

PO-274
BLOOD LETTING THERAPY IN HCV-RELATED IRON OVERLOAD - PART 1: EVALUATION OF CLINICAL, HEMATOLOGICAL AND HEPATIC PARAMETERS
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Introduction. Hemosiderosis is a metabolic, genetic or secondary, pathology characterized by tissue deposition of iron determining fibrosis and functional failure of involved organs (liver, heart and endocrine glands). If contraindications do not exist (anaemia, hypoproteinemia, cardiovascular diseases), iron overload is treated by phlebotomy. METHODS We have evaluated clinical and laboratory results obtained in 19 patients affected with hemosiderosis secondary to C hepatitis, treated in University, submitted to a cycle of weekly phlebotomy (450 mL of whole blood), in order to obtain a complete iron depletion. Tests were performed before the therapy (T0), 7 days after the treatment (T1) and after a follow-up (T2). RESULTS At T0, patients showed (mean±SE): RBC=4.9±0.1 x1012/L; HB=15.6±0.3 g/dl; PLT=197±13 x109/µL; PT=99.5±2.9%; Ferritin (F)=399.9±244 ng/mL and Transferrin Saturation (TS)=42.5±2.5%; ALT=110 U/L. The therapeutic cycle was composed by 4.3±0.5 phlebotomies (range 1-9). At T1: RBC=4.3±0.15; HB=15.0±0.36 g/dl; PLT=228±15; PT=98.5±1.7; F=51.8±4.7 and TS=16.7±2.8; ALT=76. After a follow up of 47.7±13.9 days; at T2: RBC=4.7±0.11; HB=14.4±0.4; PLT=231±17; PT=97.8±2.4; F=59.5±5.6 and TS=28.9±3.7.

PO-275
BLOOD LETTING THERAPY IN HCV-RELATED IRON OVERLOAD - PART 2: EVALUATION OF HFE GENETIC MUTATION, HCV GENOTYPE AND VIRAL LOAD
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Introduction. Iron overload is observed in about 30% of patients affected with HCV+ chronic hepatitis and it may be related with hepatic inflammation and fibrogenesis. Aim of study has been to detect an eventual reduction in viral replication (HCV-RNA) consequently to a therapeutical cycle of blood letting, performed to reduce the secondary hemosiderosis. METHODS We have evaluated the iron overload in 19 patients by assaying ferritin, plasmatic iron, total iron binding capacity (TIBC) and transferrin saturation (TS). The most frequent genetic mutations of HFE gene (C282Y e H63D) were searched; moreover HCV-RNA load and HCV genotype were also investigated. Patients have been treated by phlebotomy (400-450 mL with the mean of 5±2 blood letting) of whole blood every week. The laboratory evaluation has been performed before starting the treatment (time 0), and after a follow up of 7 days (time 1) and of 30 days (time 2) after the last phlebotomy. RESULTS Genetic mutations for C282Y were absent either in homozygosis or in heterozygosis, while 4 patients were heterozygote for H63D. Viral typing resulted: genotype 1 for 16 viruses, genotype 2 in 1 case, as genotype 3 and 4. At time 0, patients showed these values (mean ±SD): Ferritin=399±244 ng/mL, Iron=146±27 µg/mL, TIBC=350±48 micromg/mL, TS=43±6%. At sequent controls, Ferritin levels significantly decreased (91 at time 1; 59 at time 2); Iron also significantly decreased at time 1 (63), but not at time 2 (57); TIBC decreased at time 2; TS= significantly decreased between time 0 and 1 and it did not significantly go up at time 2. Viral load has not been significantly influenced by phlebotomy (time 0= 657.500 UI/mL and time 2= 655.805). Discussion. Blood letting results effective in the treatment of hemosiderosis, permitting a rapid iron depletion and its maintenance for a follow up of 47.7±13.9 days. From a clinical point of view, patients refer a generic improvement of general health conditions. Our results demonstrate that iron overload, in our patients, is certainly secondary, in fact the observed HFE mutations was unable alone to determine hemosiderosis. Moreover we have observed a total indifference of viral load, independently by viral genotype, to phlebotomy treatment, demonstrating that blood letting represents a valid therapy to prevent some complications of viral infection but it is not of aid in the treatment of primary pathologies, linked at active viral replication.
FIVE CASES OF REACTIVATION OF HEPATITIS B INFECTION AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION IN HEPATITIS B IMMUNE PATIENTS.

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Immunosuppression, including which occurs following bone marrow transplantation (BMT), potentially results in the reactivation of infections that are otherwise latent or controlled by effective immune surveillance. Although development of surface and core antibodies and loss of surface antigen following acute hepatitis B virus (HBV) infection is thought to represent clearance of the virus, evidence exists to support the possibility that the virus may remain latent within the liver. We describe five cases of reactivation of HBV infection (or reverse seroconversion) in 5 patients with prior infection and apparent clearance of HBV (i.e., test results positive for antibody to hepatitis B surface antigen [HBsAb], positive for hepatitis B core antibody [HBeAb], and negative for hepatitis B surface antigen [HBsAg]) what have developed active HBV infection (i.e., became HBsAg positive and tested positive for HBV DNA) after autologous bone marrow transplantation. From May 2001 to May 2007 we have auto transplanted in our division 111 patients (26 AML; 4 ALL; 3 CLL; 11 HD; 26 NHL; 40 MM and 1 TTP) they have been conditioned with: BEAM: 43; BuCy2: 5; BuMel: 23; MEL200: 32; others conditioning: 8 patients. In all patients we have effected the tests serologic for the hepatitis B before the transplantation and they are results: HBsAg+: 4, 4 HBsAg-/HbsAb+/HbcAb+: 34, 4 HBsAg-/HbsAb-/HbcAb+: 6; HBsAg-/HbsAb+/HbcAb: -51 and HBsAg-/HbsAb-/HbcAb: -16 patients. In total in our cohort we have 40 patients (36%) with precedent infection and apparent clearance of HBV. In all the 40 patients the HBV-Dna test has been effected and they are only 2 patients results positive for low replication. After a median of observation of 18 months after transplantation (range 3-48 months) 5 patients (12.5%) (1 AML; 1 CLL and 3 MM) have developed infection B it activates and they have reverse seroconversion (HBsAg with high HBV DNA level), 2 patients have developed an acute hepatitis and 3 a reactivation sub-clinic. In 3/5 patients has been initiated treatment with lamivudine and 1 patient (has not been treated with lamivudine) it is dead at month +4 after transplantation for the hepatitis. The risk of reactivation of HBV infection is important when considering BMT and the associated immunosuppression. The potential for poor outcomes (including chronic active hepatitis and fulminating hepatitis) following reactivation of HBV should be noted. Pre-emptive treatment with hepatitis B specific antiviral therapy may potentially have a role, but this remains to be studied. In addition, monitoring of hepatitis B serological test results and/or HBV DNA levels, as well as clinical evidence of reactivation, may allow for the early detection (and therefore treatment) of this potential very serious complication.

PO-278
PIPERACILLIN-TAZOBACTAM MONOTHERAPY AS MANAGEMENT OF FEBRILE NEUTROPenia IN PATIENTS WITH ACUTE LEUKAEMIA: A MONOCENTRIC EXPERIENCE

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Background and objective. In recent years B-lactam monotherapy as empiric approach for febrile neutropenia, has shown an efficacy similar to the standard combination therapy with an aminoglycoside. Nevertheless, this issue remains controversial mainly in selected high-risk patients. In the present study, we evaluated the impact of monotherapy with piperacillin-tazobactam in acute leukaemia and potential differences related to phases of aggressive chemotherapy, type of infections and degree of neutropenia. Methods. Febrile neutropenic patients were clinically evaluated twice: early (72 hours) and at the end of antibiotic therapy or at the recovery of each episode of neutropenia. Clinical response was classified as: success without modification (afebrile at 72 hours); success with modification (addition of an antistaphylococcal agent); failure (rescue treatment or death resulting from documented or suspected infection); not evaluable (response obtained with an antifungal in documented fungal infections or with antiviral therapy). Analysis by a Pearson Chi-Square or, when appropriate, Fisher’s Exact Test and a multivariate analysis including use of the logistic regression model was performed.

Results. From June 2003 to December 2005, 80 febrile episodes occurring in 69 patients were eligible for this study. Overall success rate both without and with modification was 75%; 34% without modifications and 41% with modification. Failure was 25%, (rescue treatment in 15% and death in 10%). Clinical response was not correlated with the phases of disease (induction, consolidation and relapse). Gram positive were responsible for 73% of microbiologically documented infections (DI). A significant correlation was documented between clinical response and type of febrile episode (p 0.068): overall success rate was achieved in 67% of DI and in 87% of FUO; success without modification was higher in FUO (47%) than in DI (25%); success with modification was higher in DI (38%) than in FUO (25%); failure was recorded in 33% of DI and in 12% of FUO (rescue treatment in 17% of DI and in 12% of FUO; death only in 8% of DI). These data were also confirmed by multivariate analysis (0.042). Patients with ANC at entry < 500/μL, were successfully treated without modifications in 82% of cases; with ANC > 500/μL in 41% of cases (p ns). Comparing these results with our previous study evaluating efficacy of combination therapy in the same patient population, no significant difference was documented in overall success rate without modification, in overall outcome during DI and FUO, in overall outcome during severe neutropenia and in failure due to death. Conclusions. Our study suggests that initial monotherapy with piperacillin-tazobactam is a reasonable option also in acute leukaemia during severe neutropenia-induced chemotherapy, although patients with documented infections are likely to require modifications.

DOUBLE EPISODE OF ACUTE HEPATITIS C AND COMPLETE CLEARANCE AFTER TREATMENT WITH ALPHA-INTERFERON POST AUTO-TRANSPLANTATION IN AML PATIENT CLINICAL CASE

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Viral hepatitis is the third most common causes of liver disease in transplant recipients and causes significant morbidity and mortality. HCV infection appears to have little short-term impact on survival after bone marrow transplantation, but is to risk factor veno-occlusive for disease (VOD) and graft-versus-host disease (GVHD) in allogeneic transplantation. Rarely a reactivation of the virus C causes an acute hepatitis after chemotherapy and/or transplantation. We describe a case of a double acute hepatitis C with complete clearance of the virus after treatment with alpha-interferon (IFN), after auto-transplant, in patient with AML. 42 year-old woman, in April 2005 diagnoses of AML (FAB M1) has been treated with DCE in induction, anti-body anti-HCV and HBV negative; in CR, the patient has effected consolidation NOVIA with har-
As for literature review, a total of 52 cases were analysed, confirming AML to be the highest risk category. Cases geographic distribution was most exclusively restricted to Spain. Clinical features were extremely variable, strongly correlating with pts immune status. Fungemia was as common in our series (2/5) as in literature data (29/52). Dissemination of infection predicted lower survival (attributable mortality rate 95% vs 23%, p<0.0001). Some successful cases using new drugs (i.e. caspofungin, posaconazole) as salvage therapy have been reported. Discussion. Scedosporiosis is extremely rare, but it represents a great problem for clinicians because of the lacking of an optimal therapy. New antifungal drugs should be considered as possible treatment options. Antifungal empiric therapy in febrile neutropenia should be targeted to agents other than scedosporiosis, such as Aspergillus or Candida, which represent the real infectious risk in haematological patients, particularly in AL.
The use of caspofungin in patients with hematological malignancies and concomitant candidemia


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Objective. To evaluate the efficacy of caspofungin in pts with hematological malignancies (HM) and candidemia Design. The study was conducted in 6 Hematology divisions; pts with clinical evidence of infection and a positive blood culture for Candida were enrolled. Patients and Results. Between 2005 and 2006,18 episodes of candidemia among patients with (hematologic malignancies (HM)) were registered. Three pts were excluded from analysis due to a premature death occurred within 48 hours from the onset of candidia. All the 15 enrolled patients (M/F; 9/6; median age 50 yo (range 19-71)) with HM (7 AML, 2 ALL; 3 NHL, 3 MM) had received chemotherapy. Five of them (33%) developed candidemia during a HSCT procedure (4 allogeneic and 1 autologous). Before the infection onset, 12 pts (80%) were neutropenic (ANC<0.5x10^9/L) for a median average of 9 days (range 2-41). Other risk factors were: CVC (93%), steroids administration (53%), mucositis (60%), diabetes (13%), Candida colonization (33%). Six pts (40%) received systemic antifungal prophylaxis (3 fluconazole,1 itraconazole,1 voriconazole) for an average of 13 days (4-46). The most frequent symptoms were: fever (100%), dyspnoea (27%), jaundice (13%), diarrhea (13%), shock (13%). Candidemia diagnosis was made after a median time of 3 days from the onset of fever; Candida species isolated were: albicans 8 pts (53%), krusei 2 (15%), talaris 2 (15%), dinei 1 (7%), tropicalis 1 (7%), haitiensis 1 (7%). Caspofungin at standard dosage was the first-line therapy in 9 pts (60%); in the other 6 pts, previous antifungal therapy (3 L-AmB, 1 itraconazole, 1 fluconazole, 1 voriconazole) was changed to caspofungin at candidemia diagnosis after no more than 48 hours of treatment. The median duration of caspofungin therapy was 13 days (range 6-26). No side effects were registered. Antifungal treatment was efficacious with remission of fever and other symptoms and negative blood cultures in 11 pts (73%). At 30 days following candidemia onset, 7 pts had died (47%), but the candidemia was responsible for the mortality in only 4 pts (overall attributable mortality 27%). Conclusions. Our data confirm the efficacy of caspofungin in the treatment of pts with HM and concomitant candidemia as well as it was reported for non hematological subgroups. Caspofungin was well tolerated also in very compromised pts.

Infectious complications in patients undergoing ASCT


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The principal complication in patients undergoing ASCT is infection. In the last decade, we have learned that the infectious risk is not equal for all bone marrow transplants and, consequently, the antibiotic therapy needs to be reasonably adapted. ASCT is considered to low risk infectious; also in our experience, the approach to the antibiotic therapy has been modulated. We have performed 552 ASCT; 410 (337 single, 73 double) have manifested a febrile episode. The percentage of transfusion ed haematological malignancies is equally distributed between the two groups: single ASCT and double ASCT, with the exception of acute myeloid leukemia present only in single ASCT group. No significant differences between the two groups with respect to the number of stem cells infused (6.5±4.2x10^6/kg in single ASCT vs. 6.0±6.7x10^6/kg in double ASCT), the time to granulocyte recovery (9.7±2.3 days in single ASCT vs. 9.5±1.2 in double ASCT) and the neutrophils < 100 mmc (6.5±5.7 days in single ASCT vs 5.6±2.7 in double ASCT ) were observed. The main characteristics of infectious and antibiotic therapies have been detailed in the Table 1. Overall, these data suggest that: 1) Monotherapy (Cephalsporins or Piperacillin/tazobactam) in 1st Line is efficacy as antibiotics association; 2) FUO is in majority between the EORTC criteria in two groups (60%, redundant data in literature), 3) Gram+ represent the principal bacteria isolated. Of note, deaths infection-related are very low (1,4%). In conclusion, febrile episodes in ASCT can be treated with antibiotic monotherapy with safety and efficacy; this approach reduce the over treatment risk without increase morbility and mortality by infection. This strategy appears to reduce them with adapted cost-benefit and reduces the risk of bacterial resistance.

Antibiotic Therapy

Table 1. Total ASCT n./% Single ASCT n./% Double ASCT n./%

<table>
<thead>
<tr>
<th>Fever/y/n</th>
<th>410/77/121 (23)</th>
<th>337 (80/80/20)</th>
<th>73 (64/41/36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EORTC criteria Classification</td>
<td></td>
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<tr>
<td>FUS</td>
<td>247 (60)</td>
<td>203 (60)</td>
<td>44 (60)</td>
</tr>
<tr>
<td>Bacteremia</td>
<td>81 (19)</td>
<td>65 (20)</td>
<td>13 (18)</td>
</tr>
<tr>
<td>Microb. Documen.</td>
<td>7 (2)</td>
<td>6 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>O/C-related</td>
<td>54 (13)</td>
<td>42 (13)</td>
<td>12 (16)</td>
</tr>
<tr>
<td>Clinical. Documented</td>
<td>24 (6)</td>
<td>21 (7)</td>
<td>3 (4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Yeast isolated</th>
<th>8 pts (53%)</th>
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<tbody>
<tr>
<td>Candida albicans</td>
<td>5 pts</td>
</tr>
<tr>
<td>Candida krusei</td>
<td>2 pts</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>1 pt</td>
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<table>
<thead>
<tr>
<th>Polymicrobial</th>
<th>2 (1%)</th>
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<tbody>
<tr>
<td>Fungi</td>
<td>2 (1%)</td>
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</table>

| Antifungal Therapy 1st line |
| --- | --- | --- |
| Monotherapy | 174 | 39 |
| Antibiotic association | 163 | 34 |

| Response to antibiotics, 2nd line |
| --- | --- | --- |
| Monotherapy | 152/213 (71%) | 125 / 174 (72%) | 27/100 (64%) |
| Antibiotic association | 126/197 (66%) | 112/163 (68%) | 23/44 (68%) |

| Mortality | 6/410 (1.4%) | 5/337 (2%) | 1/73 (1.4%) |

HBV reactivation in HBsAG negative patient after rituximab therapy for non Hodgkin lymphoma

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The chimeric anti-CD20 monoclonal antibody, rituximab, a monoclonal antibody very active in the treatment of CD20 lymphomas, especially in follicular lymphoma. This antibody have selective action on B lymphocytes, is considered a moderate immunosuppressant in terms of infection. We describe a case of reactivation HBV after treatment with rituximab in patient with NHL auto-transplanted. 60 year-old male HBsAg+; in June 1999 diagnoses of follicular NHL, the patient has been treated with chemotherapy in other division and it gets the complete remission (CR); in June 2005, in our observation, for relapse of the lymphoma, has been treated with two cycles of R-IEV and in prophylaxis with lamivudine and has been harvest CSP after the second cycle. In August 2005 the patient are in PR and in September 2003 have effected auto-transplant; has been continued treatment with lamivudine. In April 2005 (+18 months after auto-transplant) the patient suspends the treatment with lamivudine, is in CR, HBsAg+; HbsAb+ and HbcAb+; HBV-Dna <60 UI/mL. In October 2006, initial relapse of the lymphoma disease has been documented (TC small lymph nodes in lateral cervical and PTE) HBV-Dna <60 UI/mL. Begins therapy with rituximab 375 mg/mq weekly for a total of 4 doses. The patient has been revaled in January 2007, CR and PET were negative. Minimal liver enzymes increased and the reactivation HBV has been documented (HBsAg+; HBsAb+; HbcAb+ and HBV-Dna: 11x10^5 UI/mL). The patient begins new treatment with lamivudine. In May 2007 the patient is in good conditions, CR of lymphoma and the liver enzymes are normal and the situation of HBV has shown: HBsAg+, HBV - Dna: 3x10^5 UI/mL, not evident to the genotype a mutation and resistant variant, the patient continues treatment with lamivudine. Several such HBV reactivations were reported after combined rituximab and multiagent chemotherapy for B-cell lymphomas. This is a case of HBV reactivation occurring during the following rituximab monotherapy in the absence of any other immunosuppressive factor. Pre-emptive treatment with hepa-titis B specific antiviral therapy may potentially have a role, also in these patients, but this remains to be studied. In addition, monitoring of hepatitis B serological test results and/or HBV DNA levels, as well as clinical evidence of reactivation, may allow for the early detection (and therefore treatment) of this potentially very serious complication.
Myelodysplastic syndromes (MDS) are characterized by ineffective and dysplastic hematopoiesis and peripheral cytopenias. Moreover, autoimmune phenomena, mainly directed against RBC, are described in early MDS, i.e. refractory anemia (RA) and RA with ringed sideroblasts (RARS). In this study we evaluated autoimmunity in BM from 44 patients with early MDS by a method named mitogen-stimulated-direct antiglobulin test (MS-DAT), performed by stimulating BM with PMA and PHA and detecting antibodies in supernatants by competitive solid phase ELISA. Furthermore, to characterize the BM target cell of the MS-DAT positivity, we tested supernatants of positive and negative cultures on CD45-positive (myeloid cells) and CD45-negative cells (erythroblasts), separated by magnetic beads. Finally, we studied the effect of BM-MS-DAT positive culture supernatants on clonogenic activity of normal BM (CFU on methylcellulose medium). Results showed that BM MS-DAT was positive in 19/44 patients (43%). Positive patients had increased erythroblast counts and signs of hemolysis (i.e. higher reticulocytes, indirect bilirubin, and LDH, and lower haptoglobin) compared with MS-DAT negative ones. Incubation of CD45-negative (erythroid) and CD45-positive (myeloid) BM precursors with BM-MS-DAT positive supernatants, showed that the reactivity was mainly directed against autologous erythroblasts. On the contrary, MS-DAT negative BM supernatants had negligible reactivity with CD45-negative cells both from BM MS-DAT positive and negative patients. Regarding the effect on clonogenic activity, we found that addition of BM MS-DAT positive supernatants increased the overall cellularity of CFU smears along with the appearance of dis erythropoietic signs (nuclear atypia i.e. multiple nuclei), nuclear inclusions, and intercellular bridges. At variance BM MS-DAT negative supernatants had no effect on CFU smears. In conclusion, our results show an autoimmune reactivity against erythroblasts in RA and RARS patients with peripheral signs of hemolysis. BM culture supernatants from patients with this autoimmune reactivity induced normal BM progenitors to a hyperplastic and dis erythropoietic growth in vitro.

A new prognostic scoring system, called WPSS, has been proposed for MDS, based on the evidence that transfusion requirement has a prognostic role in MDS patients. This score was based on WHO diagnostic category, cytogenetic abnormalities and transfusion need. We tested these parameters in our series of 650 MDS patients with the aim of validating their prognostic value. WPSS was calculated according to Malcovati et al, by summing the scoring value of the following variables: a) cytogenetic abnormalities evaluated according to the IPSS (0 for good, 1 for intermediate, 2 for poor risk); b) WHO category (0 for RA, RARS, 5q-; 1 for RCDM and RCDM-RS, 2 for RAEB-1 and 3 for RAEB-2); c) transfusion requirement (0 for absent and 1 for regular requirement). We applied this score to 411/650 patients with available data and excluded patients without evaluable karyotype, and those with FAB RAEB-t and CMML category, not included in the WHO classification. WPSS scoring values stratified the 411 patients into five risk groups: very low (score 0, 32 patients), low (score 1, 76 patients), intermediate (score 2, 86 patients), high (score=3, 187 patients) and very high (scores>3, 30 patients). We tested this score in univariate analysis by analyzing clinical features at presentation and found a correlation with several parameters (haemorrhagic symptoms, p=0.034; platelet count, p=0.010; neutrophil count, p=0.0001; trilineal dysplasia, p=0.0001; infections at presentation, p=0.006). No statistical significances were found as to HB levels, WBC count, sex and age at diagnosis. We performed multivariate analysis on prediction of leukemic evolution (p=0.001) and on survival (p=0.0001). Time from diagnosis to first transfusion was variable, ranging from 12 days to more than 3 months (median of 1.5 months). Overall survival of different WPSS categories ranged from 57 months for patients with score 0 to 23 months for patients with very high risk score (5-6) (p=0.001). In conclusion, also in our study application of WPSS to WHO classification validating the importance of including transfusion requirement in the prognostic disease assessment.
PO-289

CLINICAL PRESENTATION OF MYELODYSPLASTIC SYNDROMES (MDS). THE EXPERIENCE OF THE PIEDMONT MDS REGISTER

On behalf of the Piedmont MDS Registry, Alessandria, Italy

Introduction. MDS patients are treated from haematology and internal medicine or geriatric institutions and information from clinical studies is not always representative of MDS population, as a consequence of a medicine or geriatric institutions and information from clinical studies website. Follow up information was updated once a year. From 1999 to and Methods.

has been active since 1999. We analyse here, for an epidemiology pur- pose, clinical and laboratory information from our data base.

Patients and Methods. Data at diagnosis were prospectively recorded through our website. Follow up information was updated once a year. From 1999 to 2006, 931 MDS patients (ps) were registered at diagnosis. 117 pts were excluded because RAEB-t (41) or CMML (76). The remaining 814 pts are the object of the present work. Results. Age was: < 60 y 111 (14%); 60-69 y 229 (28%); 70-79 y 229 (28%); > 80 y 145 (18%). Pts were clas- sified according to bone marrow blast percentage as follows: non-RAEB 503 (62%); RAEB-I 130 (22%); RAEB-II 131 (16%). 476 (58%) pts were registered from haematology and 338 (42%) from internal medicine departments. Hb (g/dl) levels were recorded in 761 pts: > 12 g/dl in 111 (14%); 10-12 in 165 (21%); < 10 in 311 (40%); < 8 in 196 (25%). Anaemia (Hb<12) was the only haematological abnormality in 40% of pts, while it was associated to thrombocytopenia (<100,000/mm³), neutropenia (<1000/mm³) or both in 20%, 9% and 16% of pts. An isolated throm- bocytopenia or neutropenia was present in only 6% and 2% pts respec- tively. The association of thrombocytopenia and neutropenia without anaemia was present in 1% of pts. No cytopenia was present in 4% of pts. Data on co-morbidities was available in 510 pts: 58 (19%) did not show any co-morbidities, while 252 (81%) presented one or more co- morbidities. The IPSS prognostic score was calculated in 599 pts: 467 (78%) low or int-1 and 132 (22%) int-2 or high. 199 causes of death were registered: leukemic evolution 56 (28%), infections 31 (16%); oth- er complications due to cytopenia 29 (14%); other age or co-morbidity related causes 83 (42%). Conclusions. MDS people have been confirmed a group of elderly frail pts. Most of them are non-RAEB at low or int-1 IPSS risk. Anaemia is the most frequent symptom and many pts die of MDS unrelated causes. Age and co-morbidities play an important role in defining the treatment strategy and an improvement in supportive care is probably even more useful than new aggressive anti-leukaemic ther- apies.

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RETROSPECTIVE ANALYSIS OF 420 PATIENTS WITH MYELODYSPLASTIC SYNDROME: MORPHOLOGICAL FEATURES AND PROGNOSTIC IMPLICATION
Travaglini E,1 Benatti C,1 Guglielmana BK,1 Della Porta MG,2 Malcovati L,2 Ascia E,2 Cazzola M,2 Invernessi R1

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In 1999 the World Health Organization (WHO) updated the FAB classification introducing bone marrow (BM) multilineage dysplasia as parameter to increase prognostic accuracy in myelodysplastic syndrome (MDS) classification; nevertheless a structured and reproducible approach for the precise definition and quantification of BM dysplasia is still lacking. Moreover the precise relationship between cytopenia and dysplasia needs to be clarified. In particular it is unclear how anemia associated with multilineage dysplasia, and bi-or pancytopenia associ- ated with unilineage dysplasia should be classified. We evaluated dyshe-板材inopietic features in BM samples from 420 MDS patients previously classified according to FAB criteria, and from 200 patients with hypore- generative anaemia. A structured cytomorphological examination was performed by counting 100 nucleated cells for each hematopoetic line- age and classifying them for their dysplastic changes and a panel of dys- plastic features showing a better sensitivity and specificity for MDS iden- tification was developed. Many of these morphological abnormalities were significantly associated with poor outcome; moreover total

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7-COLOR MULTIDIMENSIONAL FLOW CYTOMETRY (MDF) TO IDENTIFY NORMAL AND ABNORMAL MYELOID AND MONOCYTOID POPULATIONS IN MYELOCYTOMEPROLIFERATIVE DISORDERS (MPDS) AND MYELODYSPLASTIC SYNDROMES (MDS)
Gervasi F, Tortorici V, Tomasselli C, Giammanci B, Pagliaro M, Pagnucco G.

U.O. Ematologia con TMO Dipartimento Oncologia A.R.N.A.S Civico Ben- fratelli, G. Di Cristina e M. Ascoli, Palermo, Italy

In our opinion MDF represents a highly reproducible and objective way of assessing the antigen expression in myeloid or monocytoid matura- tion as well as in benign reactive or malign proliferation or in normal marrow regeneration through analysis expression of multiple antigens on a single cell to identify normal or abnormal patterns of proliferation. The aim of this study is to correlate the normal antigen expression dur- ing haematopoietic development as determined by MDF with the dys- regulation of haematopoiesis observed in MDS and MPDs. We have elaborated a 7-color immunofluorescence protocol; forward scatter (FSC) and side scatter (SSC) were collected along with 7-color antibodies combination to generate 9 parameters per cellular event. 7-color flow cyto- metric immunophenotyping was performed on flow cytometer Cyan ADFPTM (Dako, Ft Collins, CO, USA). The anti-CD45 antibody was included in each tube for gating purposes. The following panel of antibody- bodies was used in all the cases: 1. CD66-FITC/CD255a-Pe/CD45- PB/CD71-Pe-Cy5/CD11b-Pe-Cy7/CD117-APC/CD16-APC-Cy7; 2. CD15-FITC/CD13-Pe/CD45-PB/CD38-Pe-Cy5/CD3-Pe-Cy7/CD34- APC/CD16-APC-Cy7; 3. TdT-FITC/CD56-Pe/CD54/PB/CD34-Pe-Cy5/ CD10-Pe-Cy7/Mpo-APC/CD16-APC-Cy7; 4. CD64-FITC/CD14-PE/ CD45-PB/CD123-Pe-Cy5/CD33-Pe-Cy7/HLA-DR-APC/CD16-APC- Cy7. We then have been able to distinguish populations of antibodies to distin- guish normal from abnormal maturation and the patterns of expression of CD13, CD33, CD16, CD15, CD66b, and CD11b which we observe in MDSs are able to identify maturational anomalies and asinsonous antigen expression. Our results indicate that 7-color flow cytom- etry allows to distinguish normal from abnormal myeloproliferation with high sensitivity and specificity respect to the patterns described in literature with three or four color flow cytometry.

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MODIFICATION OF KARYOTYPE IN THE FOLLOW-UP OF PATIENTS WITH MYELODYSPLASTIC SYNDROME. A STUDY OF 119 ANALYSES IN 79 PATIENTS
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Introduction. The significance of changes in specific chromosomal aberrations during the follow-up of myelodysplastic syndromes (MDS) is still unknown. Methods. In 79 MDS pts aged 68 yrs (range 15-91), male/female ratio 1.5, followed for a median of 13 months (range 0-97), a karyotypic study was performed whenever pts underwent marrow
morphological reevaluation. Pts received only supportive measures, growth factors, steroids, and low-dose hydroxyurea in 4 pts with CMML. MDS was classified according to FAB and karyotypes according to IPSS risk classes. Results. A total of 79 baseline, and 119 combined morphological/cytogenetic reanalyses were performed. EAB diagnoses included 46 RA, 4 RARS, 38 RAEB, 10 RAEB-T, 10 CMML and 11 MDS not specified. IPSS karyotype risk groups included: 66 good, 26 intermediate and 27 poor. Among cytogenetic abn. used to define IPSS, del(Y) was present at diagnosis in 9 cases; at follow-up it was lost in 4, persisted in 5, and was gained in 5 cases. Del(7) was present at diagnosis in 16 analyses; at follow-up it was lost in 2, persisted in 14, and was gained in 7 cases. Del(20q) was present at diagnosis and was maintained during follow-up in 11 cases; it was gained in 5 cases, and was never lost. Del(20q) was present at diagnosis in 6 analyses; at follow-up it was lost in 5, persisted in 1 and was gained in 6 cases. Del(7) was present at diagnosis in 14 analyses; at follow-up it was lost in 2, persisted in 12, and was gained in 11 cases. Complex karyotype was present at diagnosis and 18 analyses; at follow-up it was lost in 8, persisted in 10, and was gained in 9 cases. As it is shown in Table 1, the presence of del(Y) at diagnosis predicted for a low probability of evolution; its gain at follow-up was not associated with worsening of MDS. The gain or loss of 5q- at follow-up did not impact on disease evolution. The gain of del(20q) was associated at high frequency of morphological evolution. However it occurred in 75% of cases in the context of a complex karyotype, whereas del(20q) at diagnosis carried a low risk of evolution. The persistence of del(7) or add(8) had no diagnostic impact, whereas their gain was associated at high frequency with morphological evolution. Discussion. This study shows that karyotypic changes occur frequently when cytogenetic follow-up is performed in MDS patients and definite associations can be documented between changes in specific chromosomal abnormalities and morphological evolution of MDS.

Table 1.

<table>
<thead>
<tr>
<th>FAB type changes</th>
<th>Chromosomal Abnormality</th>
<th>Better</th>
<th>Stable</th>
<th>Worse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain</td>
<td>Y-</td>
<td>3/12</td>
<td>33%</td>
<td>66%</td>
</tr>
<tr>
<td></td>
<td>Del 5q</td>
<td>5/16</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>Del 20q</td>
<td>5/12</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td></td>
<td>t(-)</td>
<td>7/23</td>
<td>0</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>B+</td>
<td>11/25</td>
<td>36%</td>
<td>64%</td>
</tr>
<tr>
<td></td>
<td>Complex karyotype</td>
<td>9/27</td>
<td>33%</td>
<td>66%</td>
</tr>
<tr>
<td>Persistence</td>
<td>Y-</td>
<td>4/5</td>
<td>40%</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>Del 5q</td>
<td>11/16</td>
<td>36%</td>
<td>55%</td>
</tr>
<tr>
<td></td>
<td>Del 20q</td>
<td>6/12</td>
<td>63%</td>
<td>17%</td>
</tr>
<tr>
<td></td>
<td>t(-)</td>
<td>14/23</td>
<td>7%</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>B+</td>
<td>12/25</td>
<td>8%</td>
<td>42%</td>
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<td>Complex karyotype</td>
<td>10/27</td>
<td>30%</td>
<td>60%</td>
</tr>
<tr>
<td>Loss</td>
<td>Y-</td>
<td>4/12</td>
<td>0</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>Del 5q</td>
<td>0/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>Del 20q</td>
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<td>100%</td>
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<td>t(-)</td>
<td>2/23</td>
<td>0</td>
<td>100%</td>
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<tr>
<td></td>
<td>B+</td>
<td>2/26</td>
<td>50%</td>
<td>0</td>
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<tr>
<td></td>
<td>Complex karyotype</td>
<td>8/27</td>
<td>74%</td>
<td>25%</td>
</tr>
</tbody>
</table>

**PO-293**

**EFFECT OF AZACITIDINE TREATMENT ON PI-PLCbeta1 EXPRESSION IN PATIENTS AFFECTED BY HIGH-RISK MYELODYSPLASTIC SYNDROMES**


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Introduction. Phosphoinositide-specific phospholipase C (PI-PLC) beta1 is a key enzyme in nuclear signal transduction, and it is involved in many cellular processes, such as proliferation and differentiation. In particular, the involvement of the PI-PLCbeta1 gene in erythroid differentiation lead us to investigate this gene in patients affected by high-risk Myelodysplastic Syndromes (MDS). It is still unclear what is the pathogenesis of the evolution of MDS into AML, even if the presence of a monoallelic and cryptic deletion of the PI-PLCbeta1 gene, as well as an impaired regulation of the PI3K/Akt axis, have been recently hypothesized to be implicated in mechanisms related to disease progression (Lo Vasco et al., Leukemia 2004; Nyakem et al., Leukemia 2006). Methods. In the present study, we performed a relative quantification, by Real-Time Polymerase Chain Reaction (PCR) analysis, on high-risk MDS patients at baseline and during treatment with Azacitidine. Furthermore, we have evaluated the expression of the PI-PLCbeta1 gene on healthy donors and the HL60 cell line, which is useful for testing the accuracy of the technology because of its low expression of PI-PLCbeta1. To analyze and quantify the levels of the two different splicing variants of PI-PLCbeta1 gene (1a and 1b), we have used a TaqMan isoformalt probe. Results. We studied 4 patients with high-risk MDS (IPSS risk high or intermediate-II) treated with azacitidine. Three of them showed a favourable response to treatment (1 complete remission, 1 partial remission and 1 haematologic improvement). Before the start of Azacitidine, all of the high-risk MDS patients showed higher levels of the PI-PLCbeta1 mRNA compared to the HL60 cell line, as expected, but lower levels compared to the healthy donors. Furthermore, MDS blasts always expressed higher levels of PI-PLCbeta1b mRNA compared to PI-PLCbeta1a mRNA. During the therapy with Azacitidine, the non responder patients showed a very low level of PI-PLCbeta1 transcripts during the whole treatment, whilst the three responder patients showed a specific activation of the PI-PLCbeta1 gene expression. Interestingly, responder patients showed fluctuations of PI-PLCbeta1 levels that could be related to the clinical status of the patients. Discussion. Our data show a correlation between azacitidine treatment and the lipid signaling pathways in high-risk MDS, and may contribute to further clarify the therapeutic activity of the drug.

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**WT1 EXPRESSION AND CLINICAL OUTCOME IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES**


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Myelodysplastic Syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by ineffective dysplastic hematopoiesis involving one or more cell lineages and characterized by peripheral-blood cytopenias and a high risk of progression to acute myeloid leukemia (AML). According to WHO classification, MDS can be classified in these following groups: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess of blasts type I and II (RAEB I and II), refractory cytopenia with multilineage dysplasia (RC+Dys), del (5q) syndrome, and MDS unclassifiable (MDS unclass). The Wilms’ tumor gene (WT1) is a tumor suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, which was originally identified for its involvement in the pathogenesis of the Wilms’ tumor. In normal peripheral blood (PB) and bone marrow (BM), WT1 expression is reported to be low and sometimes undetectable even by RT-PCR. By contrast, WT1 is highly expressed in most acute leukemias, and its level of expression is associated with the presence, persistence, or reappearance of leukemic hematopoiesis. BM samples from 36 MDS patients (16 RA, 7 RAEB I, 4 RAEB II, 4 RARS, 3 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and after 6 months. WT1 gene expression was evaluated by methods of real-time quantitative PCR (RT-PCR). At diagnosis, 22 BM samples (10 RA, 6 RAEB I, 4 RAEB II, 1 RARS, 1 deletion of 5q, 2 MDS unclass) expressed WT1 transcript amounts greater than the ranges levels of WT1 expression and after 6 months. WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. Moreover, a significant correlation was found between WT1 expression levels, blast cell percentage, and the presence of cytogenetic abnormalities. The patients received only a supportive therapy if necessary. After 6 months, 9 patients (2 RA, 5 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 expression level and a further elevation of WT1 expression after 6 months. Our results seem to confirm that WT1 gene expression could represent a useful marker in MDS to establish prognosis and progression of disease.
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A COMPLEX CHROMOSOME 3 REARRANGEMENT UNAFFECTING RPN1, EVI1/MDS1 GENES IN A PATIENT WITH AN ATYPICAL REFRACTORY CYTOPLASM WITH MULTILINEAGE DYSPLASIA.
Bernasconi P1; Dambrosio I1; Cavigliano PM1; Boni M1; Travaglini E1; Benfatti C1; Invernizzi R
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Defects of band 3q21q26 are observed in myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) with peculiar clinical features. A unique mechanism, i.e. ectopic EVI1 expression, has been suggested for the pathogenesis of these clinical entities, even if fluorescence in situ hybridisation (FISH) in our patient report a 67-year-old man who had recently undergone a surgical operation for aortic and mitral valve replacement. He presented with fatigue, weight loss and malaise and a history of mild anaemia lasting for a few years. There was no previous exposure to environmental mutagens or cancer treatments. At admission, physical examination showed pallor and slight hepatomegaly. Hematologic data were: Hb 8.9 g/dl, MCV 106 fl, reticulocytes 38×10^9/L, WBC 1.53×10^9/L with a normal differential count, platelet count 54×10^9/L. A peripheral blood smear showed remarkable red cell anisopoikilocytosis and platelet anisocytosis with many giant platelets. Bone marrow aspirate displayed hypercellular marrow. Erythropoiesis was less than 50% of bone marrow cells and showed dysplastic features. Granuloblastic line was hyperplastic with very few hypogranular and abnormally segmented neutrophils and a predominance of intermediate precursors; these cells contained many azurophilic granules in their cytoplasm but no Auer bodies and showed nuclear cytoplasmic asynchronism and bilobed or abnormally shaped nuclei. Blast cells were 4%. There were also many abnormal megakaryocytes with hypo-degranulated cytoplasm and various nuclear abnormalities. Perls reaction showed some 30% ring sideroblasts. Progenitor assay showed a normal BFU-E and colony growth. A CEP 3 and refractory cytoplas...
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**REFRACTORY ANAEMIA WITH EXCESS OF BLASTS (RAEB): HAEMATOLOGIC AND CLINICAL STUDY OF 228 PATIENTS ACCORDING TO WHO CLASSIFICATION**


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The new World Health Organization (WHO) classification subdivides the FAB RAEB into RAEB-I, with a 5-9% BM blasts and a <5% PB blasts and RAEB-II with 10-19% BM blasts and >5% PB blasts. We analysed according to this subdivision, in terms of haematological features, karyotype anomalies and prognosis, 228 consecutive patients who were diagnosed as FAB-RAEB from 1990 to 2000. One hundred thirty patients were classified as RAEB-I and 90 patients as RAEB-II. In univariate analysis we identified some statistically significant differences: median age (68 years in RAEB-I vs 78 in RAEB-II, p=0.002), median WBC count (5.4 x10^9/L in RAEB-I vs 3 x10^9/L in RAEB-II, p=0.004), median Hb level (10.5 vs 8.4 g/dl in RAEB-II, p=0.03), median platelet count (128x10^9/L in RAEB-I vs 98x10^9/L in RAEB-II patients, p=0.01). We also found a difference in rate of haemorrhagic symptoms (6% in RAEB-I vs 22% in RAEB-II, p=0.008). As to cytogentic analysis 100 patients with RAEB-I had evaluable karyotype: 20 patients showed a single chromosome change and 1 had two aberrations. Seventy RAEB-II patients had evaluable karyotype: 40 patients showed a single aberration and 10 patients presented two aberrations (p=0.02). Application of Spanish prognostic scoring system identified different categories of risk (p=0.0001), whereas Bournemouth score did not show significant differences. IPSS calculated in patients with available karyotype, was able to distinguish different risk categories in either WHO subgroups. No significant differences were found in age at diagnosis in the RAEB-I subtypes with respect to age, PB blasts, neutrophil count, transfusional requirement, infectious episodes. Leukemic transformation was recorded in 35 patients of RAEB-I group and of 43 patients in RAEB-II group (p=0.023). Median overall survival was 23.3 months in RAEB-I patients and 16 months in RAEB-II (p=0.001). In conclusion, we found that WHO classification is useful for providing risk stratification of RAEB patients, thus helping in decision making for better treatment choices.

**PO-299**

**PROGNOSTIC RELEVANCE OF RETROSPECTIVE APPLICATION OF WHO CLASSIFICATION IN 650 MDS PATIENTS DIAGNOSED BY FAB CRITERIA**


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We retrospectively re-classified by WHO system our 650 consecutive MDS cases, in order to validate the prognostic role of this classification in a unicentric series of patients. Based on FAB criteria, 196 patients were RA, 41 RARS, 242 RAEB, 74 RAEB-t and 83 CMML. The WHO classification could be applied to 479/650 patients; of 196 FAB-RA only 78 were defined as pure RA, with only anaemia and erythroid dysplasia, whereas 87 patients were re-evaluated as RCMD, for the presence of peripheral cytopenias and dysplasia in ≥10% of 2 cell lines. Twenty-nine patients had only granulocytic or megakaryocytic unilineage dysplasia, and were re-defined as MDS, unclassified (MDS-U). Of 41 FAB-RARS, 32 maintained their diagnosis, whereas 9 patients were classified as RAEB, 3 as RAEB-t and 1 as CMML. One-hundred-one patients were identified as RAEB-1 for presence of 5-9% blasts in BM, and ≤5% PB, whereas 141 patients were classified as RAEB-2 for evidence of 10-19% and of 5-19% blasts in BM and PB respectively. We tested the WHO classification in univariate analysis with regard to several clinical features at presentation. Significantly higher note of haemorrhagic symptoms (p=0.014), acute transformation (p=0.002), transfusion requirement was found in RAEB-2 and RCMD categories (107/141 RAEB-2 pts, 63/87 RCMD pts vs 59/101 RAEB-I pts). Also the application of different scoring prognostic systems, such as the Bournemouth and Spanish, showed statistical significance (p=0.001 and p=0.002, respectively). No differences were found as to sex, age, infection occurrence and cytogenetic abnormalities among various subgroups. Kaplan-Meier survival test showed significant differences, with median overall survival ranging from 55.8 months for pure RA to 22.6 months for RAEB-2 category (p=0.00001). In conclusion, the retrospective application of WHO classification to MDS patients clearly identifies prognostic correlations in various disease subtypes. Categorization of MDS entities according to WHO criteria may distinguish parameters of prognostic importance in large groups of patients with morphological similar features.

**PO-300**

**THALIDOMIDE IN MYELODYSPLASTIC SYNDROMES: A META-ANALYSIS OF PUBLISHED STUDIES**

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Thalidomide has been used to improve the anemia of patients with myelodysplastic syndromes (MDS). Its efficacy, however, is evident in a limited proportion of treated patients, while side effects may be frequent. So far, no individual clinical trial has been sufficiently extensive to provide a basis for a decision model to employ thalidomide in MDS. Therefore, we performed a meta-analysis of the seven original articles published about the use of thalidomide in MDS. Overall, this analysis included individual data from 284 patients. Thalidomide doses widely varied from 100 to 1000 mg/d and the response criteria were not uniform. Overall, average response rate was 30% (range 9-56%) and 42% (range 16-88%) on intention-to-treat analysis or considering only patients able to receive the drug for 12-16 weeks, respectively. The large majority of responses were erythroid in nature, not infrequently with relevant increase in Hb levels (up to 7 g/dl) and generally achieved within 2-3 months, without evidence of a dose-response effect. Responses were more frequently observed in patients with lower IPSS risk score, in particular in those with anemia as single cytopenia (RA/RARS vs RCMD/RCMD-RS, according to WHO classification) and with a recent diagnosis at treatment (< vs > 1 year). There was no evidence of a correlation between response to thalidomide and baseline levels of endogenous erythropoietin or transfusional support. Cytogenetic response or changes in marrow morphology were only occasionally reported. The duration of response was highly variable, ranging from three months to more than six years. Side effects, mainly peripheral neuropathy, sedation, constipation, and skin rash (but not thrombosis) were frequent, determining a very high and often early drop-out (mean 45%, range 15-67%), even in responders. However, this was almost exclusively seen in elderly patients or when thalidomide doses > 200 mg/d were employed. Based on available evidences, thalidomide remains a possible therapeutic option for selected, transfusion-dependent, lower risk MDS, if appropriately used and managed. Preferable targets appear younger patients with anemia as single cytopenia, who are not candidates for alternative approaches (such as epoetins (high levels of erythropoietin), lenalidomide (no evidence of the Sq2-cytogenetic abnormality) and high dose chemotherapy or hypomethylating agents (no blast excess).

**PO-301**

**UNUSUAL EVOLUTION OF A 5 Q- SYNDROME INTO POLYCYTHEMA VERA CHARACTERIZED BY JAK2 V617F MUTATION.**

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Introduction. The 5q- syndrome identifies a subgroup of myelodysplastic syndromes (MDS) associated to an intestinal deletion of the long arm of chromosome 5 (q31-q33) with macrocytic anemia, normal or elevated platelet count, hypobulobate megakaryocytes and a low risk of leukemic evolution. Janus kinase 2 (JAK2) is a cytoplasmic tyrosine kinase that transduces signals triggered by hematopoietic growth factors such as erythropoietin. An acquired JAK2 mutation (termed JAK2 V617F) is detectable in the majority (> 95%) of patients affected by polycythemia vera (PV), and in up to 50% of the cases of essential thrombocythemia and idiopathic myelofibrosis. The finding of JAK2 V617F outside the chronic myeloproliferative disorders is uncommon, although it has been found in a small fraction of MDS patients, either at diagnosis or at the time of leukemic transformation (Chen et al., Leukemia 2006). In particular, it has been also detected in a subgroup of patients with isolated
deletion of 5q, characterized by a high platelet count and a hypercellular bone marrow (Ingram 2006). Here we report for the first time the case of a patient with 5q- syndrome and transfusion-dependent anemia who subsequently showed an evolution into PV associated with the JAK2 V617F mutation. The JAK2V617F mutation was identified by RT-PCR followed by enzymatic digestion with BsaXI restriction enzyme. The homozygous or heterozygous state was confirmed by sequencing, performed with the same primers used for amplification on an ABI PRISM3700DNA Analyzer. Case Report. The patient, a 66 yr old male, was firstly observed in december 1998, because of mild neutropenia. The haematologic condition progressively worsened, and a diagnosis of refractory anemia, with isolated deletion of 5q and a mild thrombocytosis, was made in january 2001. The patient became transfusion-dependent 9 months later, and was started on deferoxamine on july 2005. From september 2004 the transfusional need started to decrease, and completely ceased 5 months later (Feb 9.7 g/dL). On september 05 the haematologic parameters were normal (Hb: 13.7 g/dL, WBC: 4.900/mmc; platelets 388.000/mmc). Two months later a thrombotic complication was diagnosed (deep venous thrombosis + pulmonary embolism), associated with a full picture of PV (Hmt: 55%; platelets: 659.000/mmc). At that time JAK2 V617F (heterozygous state) was detected. From then on, the patient has been treated with venesection, hydroxyurea and warfarin.

PO-302
CLINICAL EFFECTS OF 5- AZACITIDINE FIVE DAYS/MONTHLY SCHEDULE IN THREE SYMPTOMATIC LOW-RISK (IPSS: 0-1) MYELODYSPLASTIC PATIENTS
Fili C,1 Bergonzoni C,1 Skert C,1 Malagola M,1 Roccaro AM,1 Peli A,1 Capuzzi E,1 Pietrantuono G,2 Musto P,1 Russo D,1
Chair of Haematology, Unit of Blood Diseases and Cell Therapies, University of Brescia; 1Unit of Hematology and Stem Cell Transplantation, CROB, Centro di Riferimento Oncologico della Basilicata, Rionero in Vulture (Pz), Italy

Promising results have been reported by the use of nucleoside 5-azacitidine (5-Aza) in the treatment of myelodysplastic syndrome (MDS). When 5-Aza was administered at a dose of 75 mg/mq/day subcutaneous-ly for 7 days, every 28 days, it showed to be superior to supportive care, with higher response rates and reduced risk of progression to acute myeloid leukaemia (AML), mainly in the high risk MDS patients. We attempted to use an alternative schedule, 75 mg/mq subcutaneous daily for 5 consecutive days every 28 days, to evaluate its efficacy and tolera-

PO-303
THROMBOTIC RISK AND MYELOPROLIFERATIVE DISORDERS: THE GRANULOCYTES CONTRIBUTION?
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Polycythemia vera (PV) and essential thrombocythemia (ET) are considered hypercoagulable states but relatively little has been accomplished concerning the pathogenesis as well as identification of risk factors for thrombosis. We investigated the clinical and laboratory characteristics associated with the occurrence of these events in 121 consecutive patients followed between May 1986 and April 2007 (81 PV patients, median age 70 years, male/female ratio 1.39, median follow up 95 months and 40 ET patients, median age 65 years, male/female ratio 0.6, median follow up 64 months) with the aim of identifying possible predictive factors of thrombotic risk. The following variables were consid-
ered: age, gender, platelet and leukocyte count and haematocrit at the time of thrombotic event, previous history of thrombotic complication, disease duration, kind of cyto reduce therapy (hydroxyurea or pipo-

PO-304
THE RELATIONSHIP BETWEEN CLINICAL EVENTS AND JAK2V617F MUTATION STATUS IN A COHORT OF 75 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA
Dragani A,1 Malizia R,1 Iuliano Q,1 Di Marzo I,1 Patriarca A,1 Clissa C,1 Villanova I,1 Pompetti F,1 Di Nicola M,1 Esposito A,1 Davi G,1
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The Jak2 V617F mutation has been recently described in a subgroup of classical and atypical Myeloproliferative Ph- disorders. The aim of this study is sustaining the association between mutational status, ability to form endogenous erythroid colonies (ECCs), over-expression of polycythemia rubra vera 1 (PRV-1), blood count and relationship with thrombosis and abnormal bleeding in Essential Thrombocytemia (ET) patients (pts). We have retrospectively analyzed a cohort of ET pts followed at our Haematology Department. The correlation of Jak2 V617F mutation with PRV-1 over-expression and ability to form ECCs was evaluated using the chi-square test. The t-test for unpaired data was applied to evaluate the differences of Hematocrit (Ht), White Blood Cells (WBC), Platelet count (Plt) and related clinical events at the time of diagnosis and during follow-up in Jak2 V617F positive and in Jak2 V617F negative pts. The association between the mutational status and clinical events was evaluated by chi-squared test. From January 1993 to March 2007, a cohort of 75 pts (mean age 61.424.7) was followed in our Department. The follow up average time was 45.84 9 months. 32 out of 75 pts (42.6%) showed the Jak2 V617F mutation while the remaining 57.4% were Jak2
V617F negative. PRV1 was overexpressed in 21/32 Jak2 V617F positive (65.6%) and in 12/43 (27.9%) Jak2 V617F negative pts (p=0.001). EECs were found in 0.75% and in 51.1% of respectively, positive and negative Jak2 V617F ET pts (p=0.03). In the Jak2 V617F positive group, Ht was 45.3% ±2, WBC 9,61,8 and cMPD, respectively. We found a Pts mean of 620246±10/L in positive and 71800±10/L in negative Jak2 V617F group (p=ns). Finally in our cohort, 52/75 pts (42.6%) experienced thrombosis and/or abnormal bleeding. 45.7% of the positive pts showed thrombosis of 15±7 haemorrhages (n=1) and 41.8% of Jak2 V617F negative had thrombosis (n=15) or haemorrhages (n=5) (OR=1.17; IC95%: 0.42-1.17). Our study confirms that Jak2 V617F mutation is well correlated with PRV-1 overexpression and the ability to form EECs in ET. Moreover neither the mutational status nor the WBC count and Ht value affect the risk of clinical events. Finally we found a significant correlation between Jak2 V617F positive status, Ht, WBC but not with Plt count.

**PO-305**

THE INCIDENCE OF V617F JAK2 MUTATION IN BCR/ABL-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS (cMPDS): ASSESSMENT BY TWO DIFFERENT DETECTION METHODS


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Recently, recurrent and activating G to T point mutation resulting in substitution of phenylalanine for valine at position 617 (V617F) in the Janus kinase 2 (Jak2) was reported in bcr/abl-negative cMPD, including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF). Because the mutation can be present in a small proportion of granulocytic populations in MPDs, a highly sensitive detection method is required. However, the distribution pattern among cMPD is changing since more sensitive methods, other than time consuming and not always feasible sequencing technique, were developed. Aim. In this study we wanted to establish the V617F Jak2 mutation incidence in bcr/abl-negative cMPD by using two different detection methods. Methods. We analyzed 199 cases by using an allele-specific polymerase chain reaction (PCR) assay (AS-PCR). Briefly, a mutation specific forward primer containing a fluorescent tag (F) was amplified by PCR utilizing a mutation specific reverse primer. Products were analyzed using capillary electrophoresis in an automated genetic analyzer (AbiPrism 310). Subsequently, 136 cases were analyzed employing a 5 fluorescein TagMan assay (Abi 7500). This technology allows a semi quantitative measurement by which a ratio of mutation to wild-type alleles. To evaluate the sensitivity of the techniques employed, either bone marrow (BM) or peripheral blood (PB) were analyzed, while the background signal was examined by using 50 normal PB samples. Results. By AS-PCR, JAK2 V617F mutations were identified in 106/199 (55%) cases analyzed. In particular, none of sec polycytes cases (61 cases) resulted mutated, while 89% (66/74 cases) 68% (28/34 cases), 61% (11/18 cases) and 50% (6/12 cases) showed JAK2 V617F mutation in PV, TE, MIF and cMPD, respectively. Two PV cases could not definitely recognized. TagMan assay identified JAK2 V617F mutations in 118/136 (87%) cases. Again, none of the 9 sec polycytes cases resulted mutated, while only 3 out of 75 PV cases were negative. Moreover, 24/25 TE, 11/12 IMF and 13/14 cMPD showed JAK2 V617F mutations. A statistically higher percentage of the JAK-2 mutated alleles was accounted in PV (68±3.5, mean value ± sem) and MIF (64±9.3) cases as compared with cMPD (38±6.6) and ET (35±5.4). Finally, TagMan results correlated with Ht (p=0.05) and with WBC count (p=0.029) in PV cases, while the percentage of mutated alleles correlated with platelet counts in ET cases. Conclusions. Altogether, these data indicate that the JAK2 V617F mutation can be efficiently and easily detected by two sensitive techniques. TagMan technology significantly improved sensitivity in detecting JAK2 V617F mutations. Thus, JAK2 mutation screening should be considered a crucial diagnostic test in bcr/abl-negative cMPDs. Moreover, as supplementary information, the amount of mutated alleles could predict, at least in part, the different subgroups.

**PO-306**

MICROVESSEL DENSITY AND ANGIOGENIC CYTOKINE EXPRESSION IN AUTOIMMUNE MYELOFIBROSIS COMPARED WITH CHRONIC IDIOPATHIC MYELOFIBROSIS

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In patients with various degrees of isolated or combined peripheral blood cytopenias and positive markers of autoimmunity, the bone marrow (BM) assessment can reveal findings of increased reticulin fibrosis referable to autoimmune myelofibrosis (AM). This is an emerging clinicopathological entity defined by a pattern including: increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration in BM biopsies; absence of significant tear-drop poikilocytosis and leuko- erythroblastosis on peripheral blood smears; normal sized spleen; positive autoimmune markers, possibly fulfilling the classification criteria of a systemic or organ-specific autoimmune disease. The importance of neoangiogenesis has been recently established in some hematological malignancies. In particular, microvesSEL density (MVD) was demonstrated significantly increased in chronic idiopathic myelofibrosis (CIM) compared to normal controls or other myeloid disorders. Moreover in CIM, the evaluation of cellular distribution and intensity of staining for various cytokines implicated in the regulation of hematopoiesis, myelofibrosis and BM neoangiogenesis demonstrated an increased expression of basic fibroblast growth factor (bFGF) in megakaryocytes and transforming growth factor beta type 1 receptor (TGF-beta R1) in the endothelium of small blood vessels. The aim of our study was to investigate the relevance of neoangiogenesis evaluated as MVD as expression of various isoforms of angiogenic cytokines and their receptors in 14 patients with AM, using immunohistochemical staining for CD34 antigen, platelet derived growth factor (PDGF), platelet derived growth factor receptor chain A (PDGFA), platelet derived growth factor receptors (PDGFR), fibroblast growth factor receptor types 1, 2, 3 and 4 (FGFR1, FGFR2, FGFR3, FGFR4), vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor type 1 (VEGFR1), transforming growth factor beta isoforms 1, 2 and 3 (TGFbeta1, TGFbeta2, TGFbeta3), transforming growth factor beta receptor types 1 and 2 (TGFbetaR1, TGFbetaR2). The increased angiogenic activity demonstrated by MVD and expression of bFGF in megakaryocytes and TGF-R1 in the endothelium of small blood vessels was found less prominent in AM than CIM. These findings could be useful in the differential diagnosis between AM and CIM.

**PO-307**

PROGNOSTIC FACTORS IN IDIOPATHIC MYELOFIBROSIS (IM): SINGLE CENTER ANALYSIS AND PROPOSAL FOR A NEW SCORING SYSTEM

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The survival of patients with IM is known to be extremely variable, ranging from a few months to more than 20 years. Such variability needs to find some prognostic factors, in order to orientate the therapy choice in this cohort of patients. The Lille score system, proposed for patient risk stratification, seems unable to clearly discriminate between intermediate and high risk patients. The aim of this study was to evaluate risk factors in our population of IM patients and to stratify patients into risk groups. Therefore we carried out a retrospective study of 65 IM patients (43 male, 22 females, mean age 62 years; range 35-80) diagnosed between 1990 and 2000. Fifty-three cases were de novo IM, while twelve were secondary disease (polycythemia vera, 6; essential thrombocytemia, 6). Statistical analysis was performed with Logrank test, Cox test and Roc analysis. At univariate analysis the following parameters were considered as prognostic factors: age, hemoglobin level (Hb), white blood cell count, platelet count, spleen size and percentage of circulating blasts. The parameters having relevant adverse prognostic significance were Hb < 10 g/dL, and circulating blasts > 1%. The multivariate analysis considering only these two adverse prognostic factors permitted us to divide patients into 3 risk groups: low risk (0 factor), intermediate risk (1 factor) and high risk (2 factors). The median survival for each group was respectively 145, 62 and 50 months, with a very significant p value
(p<0.00005) and a better stratification than Lille scoring system. At univariate analysis Hb < 10 g/dl and circulating blasts > 1% were also able to predict the progression in acute leukemia. Obviously a wider population is needed to validate the results of this study.

Figure 1.

PO-309
SCREENING OF JAK2 EXON 12 MUTATIONS IN PATIENTS AFFECTED BY ESSENTIAL THROMBOCYTOKEMIA WITH CLONAL HEMOPOIESIS IN ABSENCE OF JAK2 V617F MUTATION

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Introduction. Essential Thrombocytemia (ET) is a Philadelphia chromosome negative disease which shows a biologic heterogeneity. JAK2 V617F mutation is present in a variable proportion of ET patients (23-72%) and is present in patients with both clonal and polycygal X-chromosome inactivation pattern (X-CIP). Recently new JAK2 exon 12 mutations have been discovered. We searched for these new mutations in ET patients with clonal X-CIP and JAK2 V617F negative. Methods. We investigated 59 ET female patients < 60 years old for the presence of JAK2 V617F mutation with allele-specific PCR according to Baxter, X-chromosome inactivation pattern (X-CIP) and we searched for new JAK2 exon 12 mutations according to Scott et al. (NEJM 2007; 358:459) in particular F537-K539delinsL, H558Q/K539L, R539L, N542-E545del in five patients with clonal hemopoiesis JAK2 V617F negative. Results. Twenty-five patients showed clonal hemopoiesis (42.4%), 20 had polyclonal hemopoiesis (35.9%) and 14 were considered uninterpretable due to constitutional skewing (23.7%). Forty-one patients of 59 (69%) showed JAK2 V617F mutation; JAK2 V617F mutation was found in 20 of 25 patients with monoclonal hemopoiesis (80%) and in 12 of 20 in the polyclonal group (60%). JAK2 V617F mutation was not correlated with the presence of monoclonal X-CIP (p=0.19). We registered 17 major thrombotic events. Thromboses were overrepresented in the monoclonal group in respect to the polyclonal one. Seventeen of the 41 patients with JAK2 V617F mutation (42%) had thromboses while no thrombotic event was recorded in wild type patients (p=0.0011), so that the absence of mutation was associated with a significant decrease in the risk of thrombosis in respect to the mutated genotype (relative risk 1.7, 95% CI 1.3-2.2). Five patients with clonal pattern of X-CIP were JAK2 V617F negative and they were investigated for JAK2 exon 12 new mutations; they showed absence of these mutations too. Finally the mechanism underlying monoclonal hemopoiesis in the absence of JAK2 V617F mutation are still unclear despite of these new JAK2 exon 12 mutations; anyhow such ET patients in our series were characterized by the absence of thrombosis.

PO-310
INFLUENCE OF THROMBOCYTOSIS ON ENDOTHELIAL AND COAGULANT ACTIVATION IN PATIENTS WITH ESSENTIAL THROMBOCYTOKEMIA

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The thrombotic risk of ET is linked to platelet hyperactivation but not to thrombocytosis. However, the thrombocytosis may cause high shear rates inducing endothelial and coagulant activation. We therefore evaluated platelets, platelet factor 4 (PF4), as indicator of platelet activation, and tissue factor (TF) and von Willebrand factor (vWF), as endothelial and coagulant markers, in 28 ET patients (15 males and 13 females, mean age 60 years) who fulfilled PVSG. Their mean duration of disease was 6 years. All patients received antplatelets. None of studied patients had inherited or acquired thrombophilia or had a history of thrombosis. Platelets were measured by automated analyser. PF4, TF and vWF were assayed by ELISA. All patients had thrombocytosis (940±297×10^3/L), high PF4 (152±69 IU/mL vs 52±2 IU/mL) (p<0.0001) and TF (174±120 pg/mL vs 81±24 pg/mL) (p<0.0001) and low vWF (36±22%) vs 101±25%) (p<0.0001). We found a correlation between platelets and TF (r=0.001) and platelets and vWF (p=0.014) whereas no correlation there was between PF4 and TF and PF4 and vWF. These results suggest that the thrombocytosis may be responsible for an endothelial and coagulant activation and that the low risk patients might be considered for a cytoreductive therapy.
PO-311
DETECTION OF THE ACTIVATING MUTATION VAL617PE OF JANUS KINASE 2 GENE IN PH1-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES

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Ph1-negative chronic myeloproliferative disorders (CMPD) represent a subcategory of hematological malignancies and are characterized by a stem cell-derived clonal proliferation of myeloid cells including erythrocytes, platelets, and leukocytes. Traditionally, Ph1-negative CMPD include Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Myelofibrosis with Myeloid Metaplasia (MMM). The Val617Phe point mutation of Janus Kinase 2 gene (JAK2V617Phe) is believed to participate in the pathogenesis of Ph1-negative CMPD and occurs in the majority of patients with PV and approximately half of those with either ET or MMM. In our institution we are following 20 patients with PV (12 M and 8 F; median age: 48 years, r.: 39-76 years), 17 patients with ET (9 M and 8 F; median age: 40 years, r.: 29-84 years) and 13 patients with MMM (8 M and 5 F; median age: 50 years, r.: 43-74 years). The diagnoses of Ph1-negative CMPD were based on the Polycythemia Vera Study Group criteria as well as bone marrow biopsy. We used the allele specific polymerase chain technique for detection of Val617Phe mutation in all 50 patients with chronic myeloproliferative syndrome. We measured Val617Phe frequency as 90% (18/20) in PV, 55% (9/17) in ET, and 38% (5/13) in MMM. We found significantly elevated hemoglobin levels and platelet count together with very low serum level of erythropoietin (measured at the time of diagnosis) in Val617Phe-positive polycythemia vera and essential thrombocytopenia patient groups compared to Val617Phe-negative patients. However, white cell count and the frequencies of splenomegaly and other complications (thrombosis, bleeding, transformation to acute leukemia) were not significantly different between the mutation-positive and negative groups. The non-invasive mutation analysis of the Janus kinase 2 Val617Phe is suitable for routine laboratory application and helps the differential diagnosis of chronic myeloproliferative syndrome. Although current informations on disease-specific prognostic relevance of JAK2V617F are inconclusive and the exact role of Val617Phe mutation testing has not yet been identified, the testing seems to be useful in cases of erythrocytoses and thrombocytoses of unknown origin.

PO-312
THE JAK2V617F TYROSINE KINASE MUTATION IN BLOOD DONORS WITH UPPER-LIMIT HAEMATOCRIT LEVEL

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By the introduction for routine tests of automated counters, it has become easy and extremely common the finding of abnormal or near normal laboratory results. During blood donations haematocrit (Htc) and haemoglobin level (Hb) are regularly tested to avoid a blood donation in the case of anaemia as this is the most frequent cause of exclusion from the donation. However it is not rare to observe a level of Hb or Htc upper or close the normal highest limit. Starting from the 1st of January 2008, we have collected 12340 donations in total from 5636 regular blood donors (total donation index 1.99) and selected, on the basis of Htc limit upper 50% for men and 46% for women, 84 males and 19 females. In this cohort, during a 1846 years donation period, the average of donations for males was 22.2 while for women was 11.4. 79 (59 males and 20 females) with normal Htc levels (median age: 48 years, r.: 39-76 years), 17 patients with ET (9 M and 8 F; median age: 40 years, r.: 29-84 years) and 13 patients with MMM (8 M and 5 F; median age: 50 years, r.: 43-74 years). The diagnoses of Ph1-negative CMPD were based on the Polycythemia Vera Study Group criteria as well as bone marrow biopsy. We used the allele specific polymerase chain technique for detection of Val617Phe mutation in all 50 patients with chronic myeloproliferative syndrome. We measured Val617Phe frequency as 90% (18/20) in PV, 55% (9/17) in ET, and 38% (5/13) in MMM. We found significantly elevated hemoglobin levels and platelet count together with very low serum level of erythropoietin (measured at the time of diagnosis) in Val617Phe-positive polycythemia vera and essential thrombocytopenia patient groups compared to Val617Phe-negative patients. However, white cell count and the frequencies of splenomegaly and other complications (thrombosis, bleeding, transformation to acute leukemia) were not significantly different between the mutation-positive and negative groups. The non-invasive mutation analysis of the Janus kinase 2 Val617Phe is suitable for routine laboratory application and helps the differential diagnosis of chronic myeloproliferative syndrome. Although current informations on disease-specific prognostic relevance of JAK2V617F are inconclusive and the exact role of Val617Phe mutation testing has not yet been identified, the testing seems to be useful in cases of erythrocytoses and thrombocytoses of unknown origin.

PO-313
RELEVANCE OF JAK2 MUTATIONAL STATUS IN ESSENTIAL THROMBOCYTHEMIA

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The pathogenesis of essential thrombocytemia (ET) is still obscure. Detection of JAK2 V617F mutation is becoming a front-line test in myeloproliferative disorders. We investigated the incidence of JAK2 and its association with other diagnostic variables in patients with ET. This is a retrospective study performed in 75 consecutive patients with a diagnosis of ET based on WHO criteria. Jak2 mutation analysis was performed on peripheral blood using allele-specific polymerase chain reaction (PCR) assay on genomic DNA and its homozygosity or heterozygosity was confirmed. JAK2 mutation was detected in 50 patients (66%). Eighteen (36%) were homozygous for the mutant allele and there were no differences according to gender. Parameters at diagnosis that were significantly associated with the presence of JAK2 (V617F) included higher counts of leucocytes (p=0.001), platelets (p=0.008), but much higher than that supposed in hospital based cohorts in previous studies (100 fold increased). The hypothesis that the prolonged myelodiploid stimulus from phlebotomies can induce inhibition of the feedback control as well as blood donations triggered the onset of a pre-existing latent primary myeloproliferative disease needs further studies to be confirmed.

PO-314
NON INVASIVE DIAGNOSIS OF PV THROUGH JAK2 GENOTYPING: CLINICAL SIGNIFICANCE OF HOMOZYGOSITY AND CD34 EXCESS

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Clinical and laboratory criteria of PVSG are not always sufficient for a diagnosis of polycythemia vera (PV). Additional criteria, namely, osteosclerotic biopsy, erythroid colonies, and serum erythropoetin levels, have improved diagnosis. According to Johansson P et al (2002), PV and AE significantly differ for red cell mass, spleen size and plasma erythropoetin, plasmatic volume and hematocrit being otherwise significantly different. Recently, Pearson added JAK2 genotyping as a further criterium. We re-classified a cohort of 123 patients affected with PV and JAK2 mutation. The mutation testing has not yet been identified, the testing seems to be useful in cases of erythrocytoses and thrombocytoses of unknown origin.
type allowed us to ameliorate diagnosis in patients with increased Hct and to further confirm that the presence of the V617F mutation is exclusive for PV consenting to distinguish PV from AE (Absolute erythrocytosis). In the light of these results, we suggest that both red cell mass measurement, serum erythropoietin and osteomiodullar biopsy still have a role for a differential diagnosis of disorders characterized by elevated Hct and negative JAK2 mutation.

PO-315

JUVENILE IDIOPATHIC MYELOFIBROSIS WITH INTRAHEPATIC EXTRAMEDULLARY HAEMATOPOIESIS AND COMPLICATED BY PORTAL VEIN THROMBOSIS: A DIFFICULT DIAGNOSIS, PROGNOSIS AND MANAGEMENT


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Introduction. We describe an unusual case of juvenile idiopathic myelofibrosis (IMF), lasting more than 20 yrs, with intrahepatic extramedullary haematopoiesis (IEH) and complicated by portal vein thrombosis (PVT). Some determinants of PVT, the prognosis and the problematic management of such rare a case are discussed. Methods. A girl was referred for isolated thrombocytosis (700000/L) since she was 2 yrs old. The bone marrow was hypercellular, with clusters of megakaryocytes, fibrosis. A watch-and-wait strategy seemed appropriate in this asymptomatic case, platelet count remaining moderately elevated in about 20 years; low-dose aspirin was finally started. Results. At 23 yrs, the thrombocytosis and the splenomegaly increased (1900000/L and 90 cm2) with leucocyte and platelet count remaining moderately elevated. Low-watch-and-wait strategy seemed appropriate in this asymptomatic case, but it was changed to hydroxyurea, because this has a major efficacy in preventing VT and reducing marrow fibrosis and splenomegaly; a long term leukemogenic effect hasn’t been demonstrated. Warfarin was started mainly as a secondary profilaxis; it isn’t clear the best duration of oral anticoagulation (long life) since IMF is an important and persistent risk factor. Our case belongs to a low risk group, according to Cervantes score, but is a clinically progressive disease (IEH, splenic infarcts and PVT). Allogeneic HSCT, that is the only real curative option for IMF, is associated with significant morbidity and mortality; so that this therapeutic option remains often controversial, like in this unusual case.

Figure 1. a) MRI demonstrated moderate splenomegaly with multiple hypochochogenic areas due to splenic infarcts. The liver showed a disomogeneous aspect with areas which were hypointense in T2 weighted and hyperintense in T1-W images without the presence of nodular lesions. a,b,c) The MRI study and the doppler color flow imaging showed in the pancreatic area numerous confluent blood vessels going towards spleno-mesenteric-portal convergence to constitute a portal cavernoma associated to multiple collateral venous circles with fibrotic transformation of portal vein (common portal vein and right and left branches), and filiform splenic vein with reduced blood flow.

The fine needle hepatic biopsy showed focal IEH (Figure 2a,b). Gastroscopy didn’t reveal ulcerative lesions or varices. A bone marrow trephine biopsy showed IMF grade II (Figure 2c,d). Hydroxyurea and warfarin were started; a research for allogeneic HSCT has been activated. Discussion. The reduced hepatopetal flow, for the sinusoidal obstruction given by the focal IEH, constituted a predisposing factor for PVT; moreover the JAK2 mutation is frequently found in PVT. Anagrelide is promising for young people; it has been changed to hydroxyurea, because this has a major efficacy in preventing VT and reducing marrow fibrosis and splenomegaly; a long term leukemogenic effect hasn’t been demonstrated. Warfarin was started mainly as a secondary profilaxis; it isn’t clear the best duration of oral anticoagulation (long life) since IMF is an important and persistent risk factor. Our case belongs to a low risk group, according to Cervantes score, but is a clinically progressive disease (IEH, splenic infarcts and PVT). Allogeneic HSCT, that is the only real curative option for IMF, is associated with significant morbidity and mortality; so that this therapeutic option remains often controversial, like in this unusual case.
uptake normalization on the spleen (but not on bone marrow). Discussion. Actually there is a high interest in chronic myeloproliferative disorders, both from biological aspects (clonality) and histopathologic field, also indicated in recent review by the introduction of new entities (WHO classification). FDG-PET/CT fusion imaging combines anatomic and metabolic data in the same scan, thus leading to a precise assessment of functional involvement of the spleen and bone marrow, visualizing the activity of hematopoietic cells and the proliferation rate in this districts, also after radiotherapy (in this case). This could be very helpful in the characterization of spleen and bone marrow role in these disorders, and probably could lead to a more precise differential diagnosis among these syndromes and to a better assessment of therapies and their effectiveness.

PO-317

THE JAK2V617F NEGATIVE ERYTHROCYTOSIS: A DISTINCTIVE MYELOPROLIFERATIVE SYNDROME?


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There is no consensus on the optimal strategy for the diagnosis of PV. The recent discovery of the recurrent JAK2V617F mutation in almost all cases of this myeloproliferative disorder offers the opportunity of redefining the current diagnosis and its classification also in a treated cohort. In our institution we have carried out a retrospective investigation of the JAK2 mutation in 40 males and 20 females referred to our centre since the last 15 years with a diagnosis of PV. DNA from their granulocytes was analysed for the mutation JAK2V617F by PCR. The patients were treated because of the risk of thrombohaemorrhagic complications: 10 with aspirin while the rest through phlebotomy and/or cytoreduction. The prevalence of the JAK2V617F mutation was 68%. We definitely diagnosed the JAK2V617F positive patients as PV and decided to reevaluate the laboratory markers of the 19 unmutated patients. All these patients JAK negative were characterized by long term follow up and by higher values for haematocrit (Hct ≥55% in males and ≥49% in females) and by an increased erythroid cellularity in the bone marrow. We compared the laboratory findings (leucocytes, platelets, EPO, Hct, HB) of the 60 patients and we did not find any statistically differences between the JAK2 positive and JAK2 negative cases. As the others figures of both groups are very similar, this result could differentiate between two groups of our patients and could suggest a different cause of exaggerated erythropoiesis. We speculate that the subgroup with erythrocytosis but without the JAK2 V617F mutation may represent another distinct entity likely with different molecular etiology.

PO-318

COEXISTENCE OF LYMPHOPROLIFERATIVE AND MYELOPROLIFERATIVE CHRONIC DISEASE: REPORT OF TWO CASES

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Introduction. The coexistence of lymphoproliferative (LPD) and myeloproliferative disorders (MPD) in the same patient is rare event. Different LPD, such as non-Hodgkin’s lymphoma (NHL), chronic lymphocytic leukemia (CCL) have been reported in association with MPD like chronic myeloid leukemia (CML). Aim. We are reporting two case of coexistence of LPD and MPD. In the first we observed the development of CML after BCR/ABL negative chronic MPD; the second developed CML after NHL. Case reports: Case 1: a 75 year old man was referred to our centre for leukocytosis (WBC 21×109/L) with immature myeloid cells in peripheral blood (PB). The LAP score was 12 (N: 20-146) and the bone marrow (BM) aspirate was hypercellular with increased percentage of the myeloid cells and 5% blasts. BCR/ABL rearrangement was negative on PCR analysis. The diagnosis of chronic MPD BCR/ABL negative was made. Three months later the patient showed an increase of the lymphocytes rate in PB with immature myeloid cells. The BM aspirate revealed 42% of lymphocytes with immunophenotype which corresponded to B-CLL (CD5, CD19, CD23 positive); FISH analysis showed +12q and del11q. Two months later the patient showed a marked increase of the leukocytosis (WBC 291×109/L) with predominance of immature myeloid cells and 18% blasts; he was treated with HU and Ara-C but died shortly after. Case 2: a 55-year-old woman referred to our centre for lymphocytosis. The BM biopsy revealed a diffuse small lymphocytic infiltration (CD20, bcl2 positive, CD5 negative). A diagnosis of lymphocytic NHL was made: the patient was treated with 6 CHOP cycles and obtained complete remission. After 14 months the patient developed progressive leukocytosis (WBC 35×109/L) with immature myeloid cells in PB. The LAP score was 22 and the BM aspirate was hypercellular with an increased percentage of the myeloid cells. The BM biopsy confirmed an increased proportion of the myeloid cells, without lymphoma cells infiltration. PCR analysis of the BM revealed e1a2 BCR/ABL rearrangement. Diagnosis of p190-CML was made and the imatinib therapy was stared. Discussion: The coexistence of MPD and LPD diseases leads to question about the origin of their cell origin. In the first case, MPD and LPD are concomitant. We can’t exclude an independent proliferation of two distinct cell lines. On the contrary, the second case seems to be a result of a bilineage manifestation of a pluripotent stem cell.
MINIMAL RESIDUAL DISEASE (MRD) ANALYSIS IN ACUTE MYELOID LEUKEMIA (AML) WITH NORMAL KARYOTYPE CARRYING NPM1 GENE MUTATIONS

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Introduction
Recently it has been reported that mutation of the Nuclear phosphoprotein (NPM1) gene, one of the most frequent molecular abnormalities in acute myeloid leukemia (AML), is most prevalent in patients with normal karyotype and is frequently associated with FLT3 internal tandem duplication (ITD) mutation. The mutations occur at exon 12 of NPM1 gene, causing a frameshift and the formation of novel C-termini causing aberrant NPM1 cytoplasmic localization.

Results
We performed MRD assay in all the patients, and analyzed the applicability of NPM1 mutations as targets for minimal residual disease (MRD) detection by real-time quantitative polymerase chain reaction (RQ-PCR).

Methods
Screening for mutation of NPM gene. For screening NPM1 mutations we amplified genomic DNA corresponding to exon 12 of NPM1 by PCR using the primer described by Falini et al (N Engl J Med, 2005). Purified PCR products were directly sequenced on a DNA sequencer (310 Applied Biosystems) Real-time quantitative polymerase chain reaction assay. gDNA was used for RQ-PCR. Specific primers and probes were used as described by Gorello et al. (Leukemia, 2006). The TaqMan Universal Master Mix (Applied Biosystems) was used for RQ-PCR. A reproducible sensitivity of 10^-4 was reached in all cases. The sensitivity was defined in accordance with the current guidelines on MRD in leukemia (van der Velden, Leukemia 2005). The telomerase gene was used as control.

Conclusion
The mean delta delta CT of undiluted DNA diagnostic samples was 3.09 (range 0.6-10). To calculate relative quantitative changes in NPM1 mutant clone during follow up we used The 2-delta delta CT method. Results
In the presented work we investigated 28 patients of de novo AML with normal karyotype. NPM1 mutations were detected in 16 patients (57.1%). Type A mutation was the only observed change. 4/16 NPM1-mutated cases were associated with FLT3 ITD (25%). We perform MRD assay in 11 cases with a follow up of at least 3 months (range 3-18 months) and we observed that NPM1 mutated clone decrease and became not relevent in all 7 patients, without a concomitant FLT3 ITD, who achieved stable molecular remission, without a concomitant FLT3 ITD, who achieved stable molecular remission.

PO-321
CLINICAL UTILITY OF IMMATURE PLATELET FRACTION (IPF) IN PREDICTING BACTERIAL INFECTION

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Introduction
Bacterial infections are a frequent cause of morbidity and mortality in hospitalized patients, usually resulting in increased peripheral leukocyte count frequently observed in surgical or Intensive Care Unit (ICU) patients. Reticulated platelet (RP), a parameter correlated with thrombopoietic recovery. A new automated method to quantify RP, expressed as immature platelet fraction (IPF), is now available by the Sysmex XE-2100 analyzer. Methods. We obtained IPF reference values (0.8-5.1, mean 2.39% on peripheral blood (PB) of 53 healthy subjects (26 females and 27 males; mean age 57 years, range 18-91). A total of 638 consecutive samples of hospitalized patients with leukocytes > 10^9/L were analyzed for IPF in a period of 3 months. Simultaneous blood cultures for bacteria were available in 78 patients (24 females and 54 males, mean age 60.8 years, range 0-90) and, out of these, 40 were positive for bacterial sepsis.

Figure 1. IPF values in negative and positive blood cultures.
Results. Microbiological agents were: Gram in 50 patients (4 E. faecalis, 4 St. aureus, 17 St. epidermidis, 3 St. hominis, 2 Str. pneumoniae), Gram in 7 patients (1 Ac anitratius, 1 Citrobacter, 2 E. coli, 1 H. influenzae, 1 Klebsiella spp, 1 Ps. aeruginosa). Two bloodstream cultures revealed multiple bacterial infections: 1 Ac anitratius+ E. faecalis, and 1 E. coli+ Klebsiella spp. Mean IPF count was statistically significant higher in patients with sepsis (5.86% range 0.5-22; 95% CI 4.1869 to 7.5481), than in patients without infection (9.65% range 0.7-10.2; 95% CI 2.0194 to 4.4911) (p<0.001) (Figure 1), while the platelet count was independent in the 2 groups. Discussion. Despite increase in leukocytes some hospitalized patients do not present bacterial complication. Therefore, this parameter is a poor indicator of infection. Our study, firstly, demonstrates the possibility to use routinely the automated IFP parameter as an indicator of infection to quickly identify subjects necessitating of strict microbiological follow-up and/or antimicrobial treatment.

PO-322
SIMULTANEOUS DETECTION OF BOTH JAK2V617F MUTATION AND EPO-INDEPENDENT ERTHROID Colonies (EECS) IN BCR/ABL-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISORDERS


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Background. We investigated both JAK2V617F mutation and Epo-independent erythroid colonies (EECS) in patients suffering from BCR/ABL-negative myeloproliferative diseases (MPDs) or other reactive conditions.

Methods. We reviewed the clinical records of patients with suspected MPDs, who visited our department of hematology over the last eight years. Diagnoses of MPDs were made based on PVSG and WHO criteria. We have been studying the JAK2 mutation since March 2005 in 68 out of 145 patients (90.6%), who were still in a follow-up period. EEsCs were detected in 74 patients (46.2%). JAK2V617F was detected in circulating granulocytes by means of a quantitative real-time polymerase chain reaction (qRT-PCR)-based allelic discrimination assay. A cut-off >0.1% of mutant alleles was established to identify positive individuals. Results. JAK-2 mutation was detected in 15 out of 20 patients (75%) with polycythemia vera (PV), in 20 out of 46 patients (43.5%) with essential thrombocythemia (ET) and in 1 out of 4 patients (25%) with idiopathic myelofibrosis (IM). Interestingly, in 68 out of 145 patients (46.9%) - evaluated as MPD-negative patients - with idiopathic or secondary polycythaemia (ES) and piaristosis, the JAK2 mutation was undetectable. EEsCs were present in 18 out of 14 patients (92.9%) with PV and in 2 out of 10 patients (20%) with ET. Moreover, only one patient with idiopathic polycythaemia was ECC-positive, while EEsCs were absent in 40 out of 41 (97.6%) patients. Finally, EEsCs and JAK-2 mutation were simultaneously assessed in 74 patients: 9 out of 14 PV patients (64.2%) were positive for both, 4 out of 14 patients (28.6%) were only ECC-positive and 1 out of 14 (7.1%) presented only JAK-2 mutation. Among ET patients, 2 out of 10 patients (20%) were positive for both; 4 out of 10 showed JAK-2 mutation. In 42 out of 43 MPD-negative patients (97.7%), no positive results were observed. Conclusions. In our retrospective study, we tried to evaluate the diagnostic impact of some biological markers, including JAK2 V617 F PCR test and EEsCs. Our study also shows a high specificity of a JAK2 PCR test in the diagnosis of MPD (almost 100%), but a low sensitivity: less than a half of ET and IM (25 and 43,5%) patients and 75% of PV patients are JAK2 mutation-positive. The combination of both markers - JAK2 PCR test and EEsCs - shows a high sensitivity and specificity (almost 100%) for diagnosing PV.

PO-323
EVALUATION OF COUTLER LH 500 RESEARCH POPULATION DATA AND THEIR USEFULNESS IN HEMATOLOGY LABORATORIES

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Introduction. modern laboratories need that haematology analysers have high reliability in both cellular counting and abnormalities signalling. This is obtained by specific alarms that help doctors in reviewing pathologica samples. Our laboratory has oncology samples and has a Coutler LH 500 analyser that offer a complete set of alarms and additional set of parameters with high utility in the slide reviewing process. The aim of this study to evaluate the Research Population Data (RPD) and their utility in myeloid and lymphoid pathologies compared with normals. Methods. Couter LH 500 obtains leukocyte differential at 125 cells per second, a multiparametric simultaneous analysis of leukocytes in their native state. The analyser evaluate the Volume, the Conductivity (size/density of the nucleus or nucleus/cyttoplasm ratio) and the light Scatter (granularity) of the cells and gives 24 RPD that refer to the mean (M) and standard deviation (SD) of the VCS measurement of Lymphocytes (LY), Monocytes, Neutrophils (NE) and Eosinophils. Cellular abnormalities are highlighted through specific alarms as well as RPD variations. 123 samples were analysed: 75 normal (used to obtain reference values of RPD), 16 myelodysplastic syndromes (MDS) (13 RA (refractory anemia); 2 RAEB (refractory anemia with excess of blast); 1 RAS (refractory anemia with sideroblast)), 32 chronic lymphoproliferative pathologies (CLP) (20 B-CLL (chronic lymphatic leukaemia), 4 atypical B-CLL, 4 B-CLL/PLL (mixed form), 1 B-PLL (prolimumphocytic leukaemia), 3 T-CLL). Slide stain: May Grunwald Giemsa. Analysier was kept controlled and calibrated following the manufacturer guidelines. Results. Reference values for RPD of LY and NE were obtained from normal samples. Analysing data of the MDS we found a significant increase of SD and decrease of M in the V, C and S of NE; this reflect morphological and/or cytoplasmic abnormalities in the myeloid cells. CLP data show a significative increase only in SD of the RPD while M were not statistically different from normals. This could be linked to the heterogeneity of the LY population. In order to focus the study on CLP, samples were divided in 3 subgroups based on RPD values. These groups show differences in the means of the RPD. LP1 (typical B-CLL with little LY) showed decrease of MV. LP2 (atypical B-CLL, B-PLL polymorphic LY with heterogeneous size) show increase of MV and MS. LP3 (atypical B-CLL, CLL/PLL, T-CLL) show increase of MV and decrease of MC. Differences in the MV appeared to be significant in differentiating the 3 subgroups. Discussion. Sample review process is based on the interpretation of the suspect flag given by the haematologist analyser, and so suspect flags and messages has to be reliable and specific for the abnormal population. This study showed how RPD values (and their differences from normals) could describe morphological characteristic and give additional information in suspect flag interpretation. Different types of pathologies show different variations of SD values of RPD with respect to normal samples, indicating abnormal heterogeneity in cellular morphology. In MDS hypolobulated and hypogranulated NE are described with decrease in MS and MC. In CLP volume size and intensity difference are clearly identified by the mean values of V, C and S based on the morphological characteristic. In particular MV seem to discriminate the different pathology. We can conclude that RPD values of pathological samples are different from normal samples and the differences and/or variations are pathology-specific. The information obtained from RPD give additional value to the blood count test and could help the doctor in addressing abnormal samples to the correct route of diagnosis. This study should be enforced with a wider collection of cases.

PO-324
CD61 EXPRESSION ON THE PLATELETS IN THROMBOCYTOPENIAS AND THROMBOCYTOSIS

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Introduction. Flow cytometric immunological counting currently provides the most accurate and reliable means to enumerate platelets, using monoclonal antibodies against membrane platelet glycoproteins. A new haematology analyzer (Abbott Cell Dyn Saphire®) provides platelets enumeration of ptls and the entry of GpIIia expression as the mean channel where fluorescent events are detected (CD61mnm). We evaluated this expression in patients with thrombocytopenia or thrombocytosis and the possibility to use CD61mnm parameter in the differential diagnosis of hematological disorders. Materials and Methods. The study population included 224 subjects: 50 normal, 109 patients affected by thrombocytopenia (48 ITP, 26 MDS, 35 Chronic Hepatitis) and 65 patients with thrombocytosis (19 TE, 46 reactive thrombocytosis). The peripheral blood samples were collected in K3 EDTA tubes and examined using CD61 FITC-conjugated mAbs and CD61mnm parameter as the mean channel of CD61-labeled events detected by FL1 subsystem. Results were analysed using Excel 7.0.
for statistical analysis of mean, comparison of means and variance. Results. The normal subjects showed a CD61mm mean value of 142.5; patients affected by thrombocytopenia showed a value of 145.8 in ITP group, 135.3 in MDS and 135.3 in Chronic Hepatitis. The differences were significant in all groups respect normal subjects and between them (p<0.0001) except for ITP patients and normals (p=0.0602), as well as between MDS and Chronic Hepatitis (p=0.5264). In thrombocytosis group, TE showed a mean of 127.2 and reactive thrombocytosis a mean of 135.4; the differences were significant either respect normal subjects and between groups (p<0.0001). Conclusions. Expression of CD61 on the platelets (CD61 mm) is different in the groups of hematological disorders that we analysed. In Thrombocytopenias, the ITP patients showed an increased expression compared to MDS and Chronic Hepatitis patients, whereas in thrombocytosis, TE showed a decreased expression versus reactive thrombocytosis. These results could help physicians in the differential diagnosis in haematological disorders characterized by abnormal numbers of platelets.

PO-325
RE-EVALUATION OF PATIENTS WITH HEREDITARY HEMOCROMATOSIS IMPROVING THE MUTATIONAL SCREENING

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Hereditary hemochromatosis (HH) is one of the most represented hereditary disorders in the western world. It is due to mutations in genes encoding for proteins regulating the iron homeostasis and it is inherited in autosomal recessive fashion. The most common mutations in genes regulating iron homeostasis are found in the HFE gene encoding for a protein (HFE) involved in the regulation of iron uptake at the cellular level (duodenum and liver) acting as an intracellular iron sensor. Two of these mutations (C282Y and H63D) are routinely screened for in patients with primary disorders of iron overload by PCR/RFLP or by real time PCR. The C282Y mutation, when heterozygous, could be associated with other mutations in the same gene, most of them considered as private or with peculiar regional distribution. In our region, two of the most frequent C282Y-associated mutations are the E168X and the W169X. Since few years ago, the mutational screening for HH was performed only searching for the presence of C282Y and H63D mutations. We recently improved the mutational screening by choosing a novel approach (reverse hybridization) for the search of 18 mutations responsible for HH (12 in HFE gene, including the E168X and the W169X, 4 in TFR2 gene, and 2 in the FPN1 gene, inherited in AD fashion) in order to look for an associated mutation in heterozygous patients and to confirm, using this mutational approach, the results previously found using the PCR/RFLP method. On the bases of the aforementioned reasons, we selected for 50 patients affected by primary iron overload, including three patients negative for both H63D and C282Y mutations, followed by our center with phlebotomy. We confirmed the results of the previous molecular investigation for 49 of the patients. The only exception was the presence of the S65C mutation in the HFE gene found in heterozygosis in a patient previously found negative for H63D and C282Y. The 16 patients heterozygous for H63D or C282Y, were confirmed also by this analysis. We can conclude that the most represented mutations related to HH in our region are H63D and C282Y, both in homozygosity or in heterozygosity or compound heterozygosity of the C282Y mutation, and the importance of the secondary effectors (age-related, environmental) or private mutations in the phenotypic expression of the H63D mutation, the most diffuse mutation in the HFE gene.

PO-326
MUTATIONAL SCREENING IN FAMILIES HARBORING PATHOGENIC MUTATIONS FOR HEREDITARY HEMOCROMATOSIS

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Hereditary hemochromatosis (HH) is a disorder due to mutations in genes encoding for proteins responsible for the maintenance of iron homeostasis. It is the most common hereditary disorder in the Western world and the clinical manifestations are variable within individuals. Two of the most common mutations found both in carriers and unaffected people are the C282Y and the H63D in the HFE gene. Due to the potential serious damage to parenchimal organs (mostly liver), pharmacological prevention of iron accumulation is recommended in individuals of pedigrees in which at least one carrier of the above mentioned mutation is affected by symptoms of iron overload. Being the penetrance of the disease variable, it has been suggested the participation of environmental factors and/or private mutations in the phenotypic expression of HH. Moreover, the most diffuse mutation in the population, the H63D seems to be transmitted as a simple polymorphism, sometimes giving rise to clinical manifestations in older people. In order to assess the presence and the penetrance of H63D and C282Y mutations, we screened for the presence of such mutations families of probands with clinical manifestation of HH and pedigrees in which the H63D mutation has been observed as occasional finding. Of the 7 families studied, one harboured the C282Y mutation, 2 were compound heterozygous C282Y/H63D, and 4 families had the H63D mutation. Clinical manifestations of HH were present in all the homozygous for the C282Y mutation and in the entire compound heterozygous (all males), while only one male with C282Y in heterozygosity presented clinical features of HH. None of the individuals had the H63D mutation were affected, except for a single case of homozygous H63D in a young male. The C282Y mutation seems to be transmitted as a pathogenic mutation, but the penetrance seems to be different even within a single family. The H63D mutation seems to be transmitted as a neutral polymorphism and such mutation is often present in a pedigree both in homozygosity and heterozygosity, not giving any clinical manifestations in older age. A population screening for HH affected to HH is not recommended, but it is important to focus the attention of pedigrees in which at least one individual present HH, bearing in mind that the variable penetrance of the mutations implies the presence of underlying private mutations which is possible to uncover using a molecular approach.

PO-327
RETRICULOCYTE RESEARCH POPULATION DATA (R-RPD): ARE THEY USEFUL IN DIFFERENTIATING SIDEROGENIC FROM EPO-STIMULATED ERYTHROPOIESIS?

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Introduction. Effective erythropoiesis can be monitored by quantitative measurement of reticulocytes which reflect bone marrow erythropoietic renewal. Coulter LH 750 performs reticulocyte analysis using VCS technology (volume, light scatter and conductivity) with NCCCLS-recommended protocol based on New Methileine-bleu staining. The results are related to different index of reticulocytes, Retics (Ret% and #), immature Retics quantification (HLR% and HLR#) and fraction (IRF) and volumetric measurement (MRV and MSVC). As further information the analyser provides the reticulocyte research population data (R-RPD) as the mean and standard deviation values of VCS data of both Retics and non-Retics populations. We evaluated the clinical utility of R-RPD in diagnosis of different clinical conditions associated with anemia. Methods. We analyzed data of: 55 Normal subjects (N) as reference population; 40 Iron Deficiency Anemia not treated patients (IDA); 161 determinations in a four months follow-up of 40 dialyzed patients treated with Epo and iron (D). Results. Normal ranges are reported in Table 1. Hb is significantly lower (p<0.0001) in IDA (9.07 g/dL) and D (10.85 g/dL). IDA has the lowest MCV and MRV values (73.36 fl and 106.92 fl) while D has the highest values (MCV 91.22 fl and MRV 127.99 fl) and the differences are statistically significant. Ret% and Ret# are higher in both D (1.3844% and 0.0664×10^9/mL) and IDA (1.9662% and 0.0741×10^9/mL) than controls (<p,0.0001), while there is no difference between IDA and D. The IRF is significantly higher in D (0.3968), while there is no difference between N (0.2954) and IDA (0.3198). There are significantly different values in Mean Retics Scatter among N, IDA and D: N= 115,71; IDA= 118,57; D= 125,16 (N/IDA=p<0.0013; N/D=p<0.0001; IDA/D=p<0.0001) (Figure 1). Moreover, the difference is also significant for both IRF (0.0241 and D: 0.0610, but it is significantly different between IDA (66.82) and N or D (p<0.0001) (Figure 2). Conclusions. Our study suggests the possibility of using the R-RPD to differentiate reticulocytes in IDA (higher Mean Ret-
ics Conductivity than healthy controls and dialyzed pa-
tients) and in patients treated with iron and Epo (higher Mean Retics Scatter than healthy controls and IDA patients).

Table 1. Normal reference values.

<table>
<thead>
<tr>
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<th>Normal</th>
<th>Mean</th>
<th>Range (2.5 - 97.5 perc)</th>
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<tbody>
<tr>
<td>HGB</td>
<td>13.209</td>
<td>12.175 - 15.7938</td>
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<tr>
<td>MCV</td>
<td>87.6720</td>
<td>81.437 - 93.6237</td>
<td></td>
</tr>
<tr>
<td>RETIC %</td>
<td>1.0784</td>
<td>0.3695 - 1.7916</td>
<td></td>
</tr>
<tr>
<td>RETIC #</td>
<td>0.0964</td>
<td>0.0187 - 0.0847</td>
<td></td>
</tr>
<tr>
<td>RIF</td>
<td>0.2954</td>
<td>0.2074 - 0.4012</td>
<td></td>
</tr>
<tr>
<td>HFR</td>
<td>0.3285</td>
<td>0.1947 - 0.6344</td>
<td></td>
</tr>
<tr>
<td>HLR %</td>
<td>0.0153</td>
<td>0.0049 - 0.0311</td>
<td></td>
</tr>
<tr>
<td>MRV</td>
<td>115.7045</td>
<td>102.1150 - 129.7687</td>
<td></td>
</tr>
<tr>
<td>MSCV</td>
<td>96.0501</td>
<td>83.7663 - 106.0650</td>
<td></td>
</tr>
<tr>
<td>V M RETIC</td>
<td>57.8000</td>
<td>51.0000 - 65.1250</td>
<td></td>
</tr>
<tr>
<td>C M RETIC</td>
<td>56.2727</td>
<td>50.7500 - 63.3750</td>
<td></td>
</tr>
<tr>
<td>S M RETIC</td>
<td>115.7091</td>
<td>107.8750 - 121.1250</td>
<td></td>
</tr>
<tr>
<td>V SD RETIC</td>
<td>14.7042</td>
<td>11.9375 - 17.3125</td>
<td></td>
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<tr>
<td>C SD RETIC</td>
<td>20.4151</td>
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<td></td>
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<tr>
<td>S SD RETIC</td>
<td>16.3900</td>
<td>14.2850 - 14.2850</td>
<td></td>
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Figure 1. Mean Retics Scatter (MRS): Dialysed patients show the highest MRS value. N=115.71; IDA=118.57; D=128.16 (N/IDA p=0.0013; N/D p<0.0001; IDA/D p<0.0001).

Figure 2. Mean Retics Conductivity (MRC): IDA patients show the highest MRC value. N=56.27; D=56.01; IDA=66.82; IDA/N and D p<0.0001).

Deletions of the chromosome 20 long arm represent a common abnor-
mality associated with myeloid malignancies, in particular with myelodysplastic syndromes (MDS) (~4%) and acute myeloid leukaemia (AML)(1-2%). Many data suggest that deletions in 20q mark the site of one or more tumour suppressor genes, the loss of which can deregulate multipotent haematopoietic progenitors. High resolution banding analy-

References


PO-328

T(20;21) DELETION ASSOCIATED WITH A 2Q DELETION IN A MDS PATIENT


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Deletions of the chromosome 20 long arm represent a common abnor-
mality associated with myeloid malignancies, in particular with myelodysplastic syndromes (MDS) (~4%) and acute myeloid leukaemia (AML)(1-2%). Many data suggest that deletions in 20q mark the site of one or more tumour suppressor genes, the loss of which can deregulate multipotent haematopoietic progenitors. High resolution banding analy-

Introduction. IgD MM accounts for 2% of myeloma subtypes and it is usually characterized by younger age and poorer outcome. In this set-

Discussion. SPE, IFE and sFLC were performed at presentation in 6 IgD-lambda MM patients and in 1 patient with heart and renal amyloidosis with IgD- lambda M-component. Five of them were also monitored after treat-
mant with different therapeutic schedules: 1 patient received Velcade, 1 conventional melphalan-prednisone (MP), 1 dexamethasone-thalido-

SERUM FREE LIGHT CHAINS (SFLC) ASSAY IN IGD MULTIPLE MYELOMA (MM)


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Introduction. IgD MM accounts for 2% of myeloma subtypes and it is usually characterized by younger age and poorer outcome. In this set-

Results. sFLC concentrations and ratio at diag-

Discussion. SPE, IFE and sFLC were performed at presentation in 6 IgD-lambda MM patients and in 1 patient with heart and renal amyloidosis with IgD- lambda M-component. Five of them were also monitored after treat-
mant with different therapeutic schedules: 1 patient received Velcade, 1 conventional melphalan-prednisone (MP), 1 dexamethasone-thalido-

Discussion. sFLC concentrations and ratio proved to be an useful tool for diagnosis and early indication of response to therapy in IgD MM patients, whereas by nephelometry and SPE it is difficult to assess the right quantification of IgD-M protein at presentation and after treatment. A longer follow-up is needed to assess the sensitivity of sFLC in detecting early disease recurrences and the efficacy of novel therapies in IgD MM patients.
EVALUATION OF NRBCS OF PERIPHERAL BLOOD IN HEMATOLOGICAL DISEASES

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Introduction. Microscopic method is the usual means to detect nucleated red blood cells (NRBCs) in the peripheral blood. Recently new hematology analyzers have been developed that allow us a fast and accurate identification and quantification of NRBCs in the peripheral blood. The Authors evaluated the presence of NRBCs in the peripheral blood of a large group of patients affected by different hematological diseases; NRBCs values were analyzed at diagnosis and during treatment. Materials and Methods. The study population included 1149 patients (572 males and 577 females, range= 1-95 years of age ) and a total of 4017 counts were performed. The peripheral blood samples were collected in K3 EDTA tubes and nrbc count was performed with an automated method based on fluorochrome – polymethine dye selective stain and lysis of nrbcs and white blood cells (Sysmex XE 2100). Results. The results are shown in the Table.

Table 1. NRBCs in peripheral blood in hematological diseases.

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<thead>
<tr>
<th>Disease</th>
<th>POS/TOT %</th>
<th>% POS</th>
<th>Median NRBC</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooley’s Anemia</td>
<td>34/35</td>
<td>97.1</td>
<td>4304</td>
<td>30-48510</td>
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<tr>
<td>Thalassemia Intermedia</td>
<td>51/61</td>
<td>83.6</td>
<td>13693</td>
<td>30-155400</td>
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<tr>
<td>S-beta Thalassemia</td>
<td>46/59</td>
<td>78</td>
<td>5670</td>
<td>50 - 20580</td>
</tr>
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<td>B-trait carriers</td>
<td>0/243</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hereditary Spherocytosis (HS)</td>
<td>0/29</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Acute Autoimmune-hemolitic Anemia (AIHA)</td>
<td>10/17</td>
<td>58.8</td>
<td>102</td>
<td>20-370</td>
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<td>Iron Deficiency Anemia (IDA)</td>
<td>0/21</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Idiopathic Thrombotic Purpura (ITP)</td>
<td>3/65</td>
<td>4.6</td>
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<td>40-60</td>
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<tr>
<td>Idiopathic Myelofibrosis (MFI)</td>
<td>13/15</td>
<td>86.6</td>
<td>445</td>
<td>20-840</td>
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<tr>
<td>Essential Thrombocytopenia (ET)</td>
<td>4/43</td>
<td>9.3</td>
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<td>0</td>
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<tr>
<td>Polycythemia Vera (PV)</td>
<td>0/30</td>
<td>0</td>
<td>112</td>
<td>42-260</td>
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<tr>
<td>Myelodysplastic Syndromes (MDS)</td>
<td>30/48</td>
<td>62.5</td>
<td>166</td>
<td>20-190</td>
</tr>
<tr>
<td>Acute Myloid Leukemia (AML)</td>
<td>20/40</td>
<td>50</td>
<td>105</td>
<td>100-260</td>
</tr>
<tr>
<td>Acute Lymphocytic Leukemia(AL)</td>
<td>7/20</td>
<td>35</td>
<td>64</td>
<td>20-160</td>
</tr>
<tr>
<td>Chronic Myeloid Leukemia (CML)</td>
<td>6/20</td>
<td>30</td>
<td>210</td>
<td>40-430</td>
</tr>
<tr>
<td>Chronic Lymphocytic Leukemia (CLL)</td>
<td>4/37</td>
<td>10.8</td>
<td>260</td>
<td>200-490</td>
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<tr>
<td>Hodgkin’s Lymphoma (HLA)</td>
<td>32/92</td>
<td>34.8</td>
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<td>20-950</td>
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<tr>
<td>Hodgkin’s Lymphoma (HLK)</td>
<td>8/30</td>
<td>26.6</td>
<td>85</td>
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<tr>
<td>Multiple Myeloma (MM)</td>
<td>19/47</td>
<td>40.4</td>
<td>74</td>
<td>20-340</td>
</tr>
<tr>
<td>Plasma Cell Leukemia (PCL)</td>
<td>2/3</td>
<td>66.6</td>
<td>114</td>
<td>20-170</td>
</tr>
<tr>
<td>Chronic Myelomonocytic Leukemia (CML)</td>
<td>2/3</td>
<td>66.6</td>
<td>165</td>
<td>20-560</td>
</tr>
<tr>
<td>MGUS</td>
<td>0/33</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anemia in solid tumors</td>
<td>32/155</td>
<td>20.7</td>
<td>108</td>
<td>20-340</td>
</tr>
</tbody>
</table>

The highest values of NRBCs, as expected, were found in severe thalassemic syndromes. In this group NRBCs values were higher in patients with splenectomy and generally correlated to the amount of ineffective erythropoiesis. The difference between the absence of NRBCs in Hereditary Spherocytosis and their presence in Acute Autoimmune-hemolitic Anemia (AIHA), both characterized by effective erythropoiesis, could be due to a much more stimulation of erythropoiesis associated with the acute AIHA. We didn’t found NRBCs in B trait carriers; this could be useful to distinguish between patients affected by mild thalassemia intermedia and B trait carriers. NRBCs were present at diagnosis in a high number of patients affected by CML and MFI, followed by severe aplastic anemia, that one of the most common alteration in Acute Myeloid Leukemia (AML), FLT3-ITD and other mutation involving FLT3 gene are adverse prognostic factors, even if associated with other favourable index as Nucleophosphomin cytoplasmatic dislocation (NPM+) or Core-Binding Factor (CBF) mutations. In FLT3-ITD positive AML, qualitative PCR analysis usually shows a wild type FLT3 gene associated to a mutated one. This alteration can be confirmed by the gene sequencing. Hereafter we describe a case of AML which shows homoyzous FLT3-ITD associated to NPM+ with an extremely adverse clinical presentation. Case report: a 60 years old man was referred to our division for de novo myelo-monocytic AML. Clinical features at the onset showed an high blast count in peripheral blood (168,000 blasts x10^9/L), septic state starting from dental abscess, initial respiratory distress and disseminate intravascular coagulation. Cytogenetic analysis revealed a normal 46-XY karyotype, PCR analysis showed homoyzous FLT3-ITD mutation, gene sequencing confirmed the presence of mutated streams alone with tandem duplication in exon 14. Type A NPM mutation was also present. A prompt procedure of leukapheresis was performed in order to reduce WBC count, followed by a cyto-reductive chemotherapy with low-dose of cytarabine. Four days after, Idarubicin and Etoposide were associated with standard doses. But the progressive worsening of pulmonary distress needed ventilatory assistance, the extension of septic state and a multi-organ failure caused a fatal outcome 28 days from the onset. Discussion: this case is an extremely rare situation of homoyzous gene mutation in AML. Usually one or more gene alterations in heterozygous state are sufficient to determine an abnormal cell growth in acute leukaemia. Otherwise, in chronic subset such as myeloproliferative disorders (MPD) homozyous status of JAK2(V617F) mutation, derived from mitotic recombination or one-hit inactivation event, causes an extremely shortened life span. The specificity of our case is the demonstration of an homoyzous mutation into a de novo AML; we do not know weather the homoyzous state derived from a previous asymptomatic heterozygous FLT3-ITD mutation but mutation data still can suggest as this particular genotype seems to be associated with an extremely adverse presentation.

A NEW CYTOGENETIC ABNORMALITY, T(11;4) (P22;P16) IN AML M4 PATIENT AFTER CHEMOTHERAPY TREATMENT

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Cytogenetics studies at the diagnosis provides the most important prognostic information in young adult AML. Here we report the case of a 16-year-old female patient with de novo AML M4eo according to the FAB classification, whose blasts showed the following positive antigens: MPO (20%), CD14/CD11 (58%), CD34 (21%), CD33/HLA-DR (59%), CD13/CD117 (17%), lysozyme (22%). Conventional cytogenetic analysis performed at diagnosis revealed the karyotype 46, XX, inv(16) in most of the examined bone marrow cells, thus the clinical outcome was that one of low risk AML. The patient underwent conventional chemotherapy with first course of cytarabine 100 mg/sm for 10 days, Daunorubicin 50 mg/sm for 3 days, and Etoposide 100 mg/sm for 5 days and obtained complete remission (CR<2%). The second course, after 1 month, consisted of Cytarabine 500 mg/sm twice a day for 6 days, Daunorubicin 50 mg/sm for 3 days and lenograsit until PBSG har-
The main differences are found in the spectral region between 1500 – 1650 cm⁻¹, which is strongly affected by the Fe spin state and, therefore, by the oxygenation conditions of the cell. In particular, the relative band intensity of the Raman features at 1547 and 1581 cm⁻¹ is quite sensitive to the RBC healthy state. By repeated measurements on both classes of RBCs, it was found that the ratio of the strengths of these bands is a significant parameter for the diagnosis of Thalassaemia. The obtained results are quite promising for the development of new clinical protocols for the diagnosis of Thalassaemia.

**PO-334**

**IDENTIFICATION OF COMPLEX CHROMOSOME REARRANGEMENTS IN A PATIENT WITH ACUTE ERYTHROIDBLASTIC LEUKAEMIA (AML-M6) BY USE OF FISH AND SKY**

Donadio F,1 Lionello A,1 Altieri V,1 Gentile G,1 Fotino A,2 Sementa A,1 Bernardini L,1 Zatterale A,1 Cantore N²

¹U.O.C. Genetica, P.S.I. Elena d’Aosta, ASL Napoli 1; ²S.C. Ematologia e, Roma, Italy

Erythroleukaemia (AML-M6) often exhibits complex karyotypic abnormalities associated with a poor prognosis. Most patients (47%) show hypodiploidy while partial or entire monosomies represent 56% of abnormalities. Chromosomes 5 and 7 are most frequently involved, followed by chromosomes 5, 16 and 21. Unbalanced abnormalities are more frequently found than balanced. We report a case of a 62-year-old woman with anaemia and mild leukaemia. Bone marrow (BM) aspiration demonstrated 65-70% myeloid blast cells, erythroid hyperplasia and dyserythropoiesis. Bone marrow biopsy showed hypercellularity, prominent erythroid hyperplasia/dysplasia and clusters of myeloid blast cells, decreased number of myeloid precursors and megakaryocytes. Standard cytogenetic analysis performed on BM identified in all metaphases partial monosomies of chromosomes 7 and 16, and a chromosome 19 derivative in association with a complex ring. Subsequently FISH and SKY were performed to better identify the rearrangements of BM cells. Using both conventional and molecular cytogenetic techniques we were able to define the karyotype as: 45,XX,-7,add(9)[q34.1]-16,der(19)[dup(19)](q) [t(7;19)[q11.23;q13.4]], der((16;19)]. This is the first case of AML-M6 with multiple rearrangements of chromosome 19, which is here implicated both in a derivative chromosome from unbalanced translocation with chromosome 7, and in a ring involving chromosomes 16 and 19. A review of the literature reveals recurrent rearrangements involving the chromosome 19, which breakpoints are located in 19q13.1, but the possible role of genes on chromosome 19 in erythroleukaemogenesis can only be surmised.

**PO-335**

**A CONCOMITANT T(4;11) AND A T(1;19) IN A PATIENT WITH BIPHENYLCYTIC ACUTE LEUKAEMIA**


Division of Hematology and Transplants, University of Siena, Italy

**Background.** A minority of acute leukemias have features characteristic of both myeloid and lymphoid lineages and for this reason are designated as mixed lineage or biphenotypic leukemias (BAL). Some cytogenetics abnormalities are known to be specifically associated with this type of leukaemia, i.e. abnormalities involving the MLL gene at 11q23 (also known as ALL1, HTRX, HRX), which is involved in several translocations with different partners. In particular t(4;11)[q21;q23] is a recurrent abnormality in acute lymphoctic leukaemia (ALL), but may be present also in acute myelocyctic leukaemia (AML) and in BAL, representing a clear poor prognostic factor. The MLL gene is usually disrupted upstream of the region coding for the zinc finger sequence motifs of the protein product. The 3' sequences are translocated or lost and the 5' regions are juxtaposed to the coding sequences of the genes of its translocation partners. In addition, about 5% of ALL and 20% of pre-B ALL show a t(1;19)[q23;p13] that is associated with adverse prognostic features, mostly in children and young adults. The N-term transcriptional activation domains from E2A at 19p13 fuse to the Hox cooperative motif activated by the domain of T-term PBX1 at 1q21. PBX1 is a potent transcriptional activator. Patients and Methods. A 61-year-old male patient presented with a BAL and a concomitant t(4;11)[q21;q23] and t(1;19) (q23;p13). The patient was referred to our division because of leukocytosis, anaemia and thrombocytopenia. Physical examination showed a mild hepatomegaly. A peripheral blood sample showed Hb 9.6 g/dL, PLT 60 × 10⁹/L and WBC 32 × 10⁹/L, with 6% neutrophils, 30% of small lymphocytes and 64% minimally differentiated blasts at blood smear examination. Analysis of bone marrow aspirate evidenced 79% of leukemic cells that showed positivity for myeloid antigens such as CD15 and myeloperoxidase (MPO), while CD13, CD33 were negative. Also a positivity for lymphoid antigens was observed, i.e. DTD, CD22, CD19, cytoplasmatic IgM. Cytogenetic analysis was performed with a Wright-Giemsa banding technique on bone marrow aspirate. The karyotype...
showed: 46,XY [16 cells], 46,X,Y, +der(19) t(1;19)(q23;p13), t(4;11)(q21;q23) [4 cells]. Fish analysis confirmed translocation of the MLL gene in 90% of 200 nuclei independently scored by two different observers. RT-PCR confirmed a fusion transcript for AF4-MLL, while the E2A-PBX fusion transcript for t(1;19) was not present. The patient began an intensive induction chemotherapy regimen (L20 modified) including vincristine 1.4 mg/sqm , cyclophosphamide 1000 mg/sqm , doxorubicin 20 mg/sqm but unfortunately died for septicemia due to Pseudomonas Aeruginosa on day 19. Discussion. Even though t(4;11) has been reported in 539 cases in the literature and t(1;19) in 144 cases according to the Mitelman database, this is to our knowledge the first patient carrying both translocations. In our patient MLL was confirmed translocated by Fish and RT-PCR, while the E2A-PBX transcript deriving from the t(1;19) was not found. Our case appears as 3/4 of the cases described in the literature with a t(1;19) having an unbalanced form of this translocation with 2 normal chromosomes 1, a der(19) and a normal chromosome 19. The peculiarity of this case is due to the absence of the E2A-PBX transcript at the molecular level. It is possible that the breakpoint does not involve as usual exons 14 or 14 of E2A and that a variant transcript is present. Although our patient died soon for a sepsis, we cannot draw certain conclusions about prognosis. In conclusions, we describe for the first time a case of BAL with concomitant t(4;11) and t(1;19). Further molecular studies are needed to better delineate a possible variant breakpoint on chromosomes 1 and 19 and a variant transcript of t(1;19).


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Introduction. Indifferentiated cells are originated from incontrolled and abnormal proliferations of blasts from leukemogenesis processes. Specific tumor sequences as rearrangement and mutations are utilized in Frankfurt. Provided from Instituts für Pharmazeutische Biologie JWG Universität München. Molecular rearrangements in patients report a long overall and event free survival. Materials. We have analyzed 83 cases of Acute myeloid Leukemia, 57 from Cancer Hospital A Businco Asl 8 Cagliari and 26 cases from Cancer Institute S.O.L.C.A of Cuenca (EC). Methods. All cases were evaluated with RT-PCR procedure, t(4;11), t(9;11) rearrangements and rare translocations of MLL were performed with probes and procedure kindly provided from Instituts für Pharmazeutische Biologie JWG, Universität Frankfurt. Results and Conclusions. None of the 83 cases observed were positive for MLL/AF4 and MLL/AF9 molecular rearrangements. Specific new diagnostic method may provide correct evaluation of derivatives AF4 and AF9 with relative wide-type and complete absence of aspecific PCR products.

INCIDENCE OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE IN WELL DEFINED HOSPITALIZED AREA: M. G. VANNINI HOSPITAL. – ROMA


UCSC Roma, Italy

It is known that monoclonal gammapathy of undetermined significance (MGUS) is defined by the presence of a monoclonal protein in persons with no features of multiple myeloma or other related malignant disorders such as Waldenström's macroglobulinemia, primary amyloidosis, B-cell lymphoma, or chronic lymphocytic leukemia. Patients with MGUS have a serum monoclonal protein concentration lower than 3 g per deciliter, fewer than 10% plasma cells in the bone marrow, and no clinical manifestation related to the monoclonal gammapathy. Recent study population based involving a county in Minnesota reports that the prevalence of this condition was 3.2% in persons older than 50 years of age and that was higher in men than in women (4.0% vs 2.7% among persons 50 years of age or older). With the enhancement of the sensitivity of the analytical techniques the report of MGUS increase and make physiotherapeutical ways of solve the problem of further examinations. We retrospectively study a multiethnic population in a well defined hospitalized area using Hydrasis electrophoretic system by Sebia Italia from 01/01/2006 to 31/12/2006 collecting data by the computerized system of the our laboratory (Italnoema s.r.l.). Among 10.042 agareso gel electrophoresis performed in our laboratory (6556 for hospitalised patients and 3486 out-patients), 78% were older than 50 years of age and 55% were female. We report 462 patients (excluding those with repeated analysis) having suspected monoclonal component including any discrete band of monoclonal protein or thought to have a localized band (4.6%). Among the total electrophoresis performed, 2.1% of suspected monoclonal band reported were female and 2.5% were male; 4.5% were older than 50 years of age and 4.5% were patients whose Italian ethnic group was known. The relatively high incidence of MGUS in our studied population has implications for any screening programs or preventive strategies that could be developed to reduce mortality from myeloma.

EVALUATION OF IRON CONTENT OF SERUM FERRITIN IN PATIENTS WITH HYPERFERRITINEMIA

Ricera BM, Rossi C, Venditti D, Marino M, Spada PL, Alimonti A, Storti S, De Sole P

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We investigated if Ferritin is differently charged with iron in various clinical settings evaluating the Iron Content of Ferritin (ICF) in patients with high serum-Ferritin (s-F). We examined three groups of hyperferritinemic patients (Fe2000ng/ml; Mx200ng/ml): Uremic patients treated with haemodialysis (A n=22), Septic patients recovered in Intensive Care Unit (B n=16), outpatients of our Haematology Unit (C n=30). This last group is heterogeneous, including patients with Primitive Iron Overload (IOV) e.g. Hereditary Hemoschromatosis (HH n=8) and others with Dysmetabolic Syndrome (DMS), 2 with Chronic Liver Disease (CLD) HCV+, 2 with Myelodisplastic Syndrome (MDS), 1 with Congenital Dyserythropoietic Anemia (CDA). Only two patients were regularly transfused (1 TI, 1 SCA). Complete Blood Count, serum-iron (s-I), Total Iron Binding Capacity (TIBC), s-F and other hematchemical parameters such as ALT, AST, gamma-GT, IL-6 were measured; TSI was calculated. ICF was assayed according the Yamaniishi H et al technique (Clin Biochem 2002), partially modified. The following Table 1 shows the main results of the study. The s-F doesn't differentiate the three groups while TSI shows a different and statistically significant value in each of the three: the lowest in septic patients (inflammation effect) and the highest in haematological patients. However, ICF is statistically different in the three groups; therefore we considered separately all patients who had ICF as a consequence of Erythroid Disorders (ED) (6 TI, 2ASC, 2MDS, 1 CDA n=11); the second group includes the Primitive and Metabolic
IOV (8HH, 9DMS, 2CLD n=19). The two groups don’t differ for iron balance parameters, only the ICF is higher in ED group. In conclusion, the common parameters of iron balance roughly indicate the body iron content. In particular, it is well known that s-F can be influenced by many variables (inflammation, liver cytolisis etc). Moreover, the ICF evaluation gives a new information: the s-Ferritin doesn’t carry the same amount of iron in any clinical setting. In septic patients who have the lowest TSI, the s-F carries the largest amount of iron. In the end, ED patients have a severe state of IOV comparable to the one of HH and DMS for classical parameters of iron balance. This state is more severe according the s-F ferritin. In only 2 patients out of 11 the IOV is influenced by transfusions. This observation should induce to pay more attention to the IOV state in ED patients not transfused.

## Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group A</th>
<th>p</th>
<th>Group B</th>
<th>p*</th>
<th>Group C</th>
<th>p*</th>
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<tr>
<td>TSI%</td>
<td>28.9±2</td>
<td>0.056</td>
<td>23.2±20.2</td>
<td>0.0004</td>
<td>18.2±18.7</td>
<td>&lt;0.0001</td>
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<tr>
<td>F ng/mL</td>
<td>789.7±900</td>
<td>ns</td>
<td>766±736.9</td>
<td>NS</td>
<td>732.9±691</td>
<td>NS</td>
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<tr>
<td>ICF pg/ng</td>
<td>27.1±16.7</td>
<td>0.0003</td>
<td>64.8±35.7</td>
<td>0.02</td>
<td>45.8±27.9</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-6 ng/L</td>
<td>6.9±18.9</td>
<td>&lt;0.001</td>
<td>169.5±149.8</td>
<td>&lt;0.0001</td>
<td>3.39±12.3</td>
<td>0.004</td>
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</table>

p was calculated with Wilcoxon Mann Whitney between A-B; p* B-C; p* A-C

Introduction. In the last year, the issue of cardiotoxicity of imatinib mesylate (IM) was on focus. Emerging data seem to deny an increased risk of cardiac events in patients treated with IM, which is the frontline therapy in chronic myeloid leukemia (CML). B-type natriuretic peptide (BNP) is released by the heart in response to myocardial tension and is considered an accurate test for the diagnosis of heart failure. The measurement of BNP in the serum is a rapid and easy tool for evaluation of ventricular function, also in asymptomatic patients. Methods. We have measured BNP level in 50 patients (35 males and 17 females) with chronic phase CML treated with IM at our Institution. BNP was measured using a direct chemiluminescent sandwich immunoassay: the analytical range extends from 0 to 5000 pg/mL, with a sensitivity <2 pg/mL. Normal range is as follows: <100 pg/dL. Median age was 59 (range: 23–82). Median duration of IM therapy was 38.5 months (range: 1–81). IM mean daily dose was 404 mg (SD±121). Thirty three patients (66%) received 400 mg/day, 13 (26%) a lower daily dose (200 mg in 1 case, 500 mg in 12) and 5 patients (10%) had higher IM doses (600 mg in 2, 800 mg in 3). Results. The mean level of BNP in the whole population was 22.0 pg/ml (SD ±26.4); only two patients had values >100 pg/mL. There was a linear correlation between age and BNP levels (t-value=3.850, p=0.0005). Nine out of 24 (37%) patients aged ≥60 had BNP >22 pg/mL, compared to only 1/25 (4%) in the cohort <60 years old (χ²=19.7, p=0.0001). BNP level was not affected by IM daily dose (<400 mg = 34.1±28.6, 400 mg = 15.5±26.2, >400 mg = 30.5±9.3) or by therapy duration (<36 months = 25.2±30.4, ≥36 months = 19.4±22.6). Considering cardiovascular risk factors, 18 patients (36%) had hypertension, while diabetes and hyperlipemia were present in 2 and 4 cases, respectively. Patients with hypertension had higher levels of BNP (34.9±35.1 vs 14.8±16.5, p=0.03), despite an equivalent IM dose (378±81 vs 419±138 mg) and treatment duration (59.5 vs 30.5 months). No patient experienced major cardiac adverse event during IM therapy. Discussion. Imatinib therapy does not cause an increase in BNP levels. This gives an indirect confirm to the cardiac safety profile of the drug, as indicated also by the lack of major cardiac toxicities in our patients. BNP levels were affected by hypertension and by advanced age, but the latter could be a bias due to a higher incidence of hypertension in the elderly cohort.

## Po-338

**MicroRNAs as the Target of Deletions on Der(9) in Chronic Myeloid Leukemia**


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Introduction. Deletions on der(9) are associated with chronic myeloid leukemia(CML) in 15-18% of cases. To date, the biological significance of this genomic loss in the pathogenesis of CML is unknown. The most plausible hypothesis is that the loss of a tumor suppressor gene may confer a proliferative advantage to the Philadelphia-positive clone. On the other hand, it has now become evident that microRNAs (miRNAs) play an important regulatory role in some hematological malignancies. To investigate the presence of miRNAs within the genomic regions lost on der(9) we analyzed 60 CML patients with der(9) deletions. Methods. Genomic characterization of the deleted sequences was performed by fluorescence in situ hybridization (FISH) using a contig of DNA clones; the miRBase (http://microrna.sanger.ac.uk/) was queried to assess the presence of miRNAs in the der(9) deleted genomic regions. Results. FISH experiments showed that the genomic loss on der(9) of the 9 (centromeric to ABL) and 22 (telomeric to BCR) chromosome sequences ranged

**Chronic Myeloid Leukemia**


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from 260 Kb to 54 Mb and from 230 Kb to 12.9 Mb, respectively. Consid-
eration of the miRbase revealed that in 16 (27%) patients there was loss of miRNAs mapping on chromosome 9 whereas no known miRNAs were mapped on the deleted genomic sequences belonging to chromo-
some 22. Moreover, 4 cases with a complex t(9;22) rearrangement and del(9) deletions showed loss of the miRNAs sequence also on the third derivative chromosome (4p16, 7p14, 13q14, and 11q13, respectively); among them, only in one case the loss of miRNAs on the third deri-
utive was not associated with the miRNAs deletion mapped on chromo-
some 9. The most recurrent miRNAs deleted on del(9) were mir-219-2 (deleted in 100% of cases) and mir-199-b (lost in 67% of cases). It is noteworthy that mir-219-2 neighbors and overlaps CpG-islands, sug-
gesting a potential role of this miRNA in CpG-island methylation. Con-
clusions. Experimental studies indicate that miRNAs can function as tumor suppressor genes or as oncogenes. In fact, in chronic lymphocyt-
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ciated with miRNAs loss may shed new light on the significance of genominc sequences loss. Further studies are needed since it is known that some microRNAs may have as many as a few thousand targets, so pre-
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References
MOLECULAR RESPONSES OF EARLY CHRONIC PHASE MYELOGENOUS LEUKEMIA PATIENTS IN THE IMATINIB ERA: INTERFERON ALPHA MAY STILL HAVE A ROLE?


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Introduction. Thanks to its striking effectiveness, imatinib (IM) is the established first line treatment of early chronic phase (ECP) chronic myeloid leukemia (CML) patients (pts), where a complete cytogenetic response (CCgR) is achieved in more than 80% of pts. Nonetheless, the achievement of an major molecular response (MMolR), which correlates with a more durable cytogenetic response and better progression-free survival, remains a focus of newer regimens. To explore a possible role of Interferon-alpha (IFNalpha) in this regard, we compared the molecular response of 569 ECP pts who achieved a stable CCgR with IM 400 mg (309 treated with IM alone and 60 treated with IM and IFNalpha).

Methods. Pts were monitored for conventional cytogenetic and molecular response every 6 mos. Peripheral blood samples were centralized in Bologna (RQ-PCR, Bcr-Abl/Abl x 100 - Taqman). Major molecular response (MMolR) was defined as BCR-ABL/ABL% less than 0.05, whereas undetectable BCR-ABL transcript levels was defined as BCR-ABL/ABL% less than 0.001.

Results. 76 ECP CML pts (enrolled in the 011 study of the ICSG) were treated with IM and a variable pegylated IFN-alpha dose (50, 100 and 150 microg/wk); 60 are in stable CCgR and evaluable for molecular response. 358 ECP pts (enrolled in the 022 and 025 study of the ICSG) were treated with IM 400 mg; 309 are in stable CCgR and evaluable for molecular response. Pts were equally distributed by Sokal risk in both groups. The frequency of MMolR increased during follow-up in both groups, but the rapidity of achievement and the quality of the molecular response was higher in ECP pts treated with IM and IFNalpha than in IM pts. The median follow-up for the 45 living pts is 66 mos (55-73). 189 pts (93%) were previously treated for CML in chronic phase (CP) for 3 to 26 mos (median: 6), while 14 had the disease onset in AP/BC. Of 111 pts treated in AP, 107 pts (96%) showed a return to CP and 74 pts (70%) obtained also a complete hematologic response, which lasted at least 4 weeks in 68 (61%) pts. A complete cytogenetic response (CCgR) was achieved by 15 (14%) pts, in 2 to 15 mos (median: 4) with a major CgR rate of 25%. CCgR was subsequently lost by 8 pts after 3 to 50 mos from its first achievement, with a median CCgR duration of 21 mos. Overall survival decreased from 86% to 71% and 49% at 6, 12, and 36 mos, but remained stable thereafter (37% at 66 mos). The median survival time was 36 mos. Of 92 pts treated in BC, 57 pts (62%) returned to CP and 24 pts (26%) obtained also a complete hematologic response, which lasted at least 4 weeks in 22 pts. A complete cytogenetic response (CCgR) was achieved by 8 (9%) pts, in 2 to 11 mos (median: 4), with a major CgR rate of 20%. CCgR was subsequently lost by all but 2 pts after 2 to 12 mos from its first achievement, with a median CCgR duration of 7 mos. The median survival time for BC pts was 7 mos, with 8% of pts alive at 66 mos. Conclusions. IM 600 mg has a substantial activity when used as a single agent in pts with CML in AP and BC. Pts treated in AP showed a significantly higher hematologic and cytogenetic response rate, as well as better OS than BC pts. Overall, OS remains stable after 36 mos of therapy, indicating that IM therapeutic effect is durable over time. Acknowledgments. COFIN 2003, FIRB 2001, AIRC, CNR, Fondazione del Monte di Bologna e Ravenna, European LeukemiaNet, AIL.

IMATINIB 600 MG IN THE TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA IN ACCELERATED PHASE AND IN BLAST CRISIS. RESULTS OF A MULTICENTRIC PROSPECTIVE STUDY OF THE GIMEMA WORKING PARTY OF CML IN THE LONG TERM


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Introduction. The efficacy of Imatinib (IM) on long-term survival in accelerated phase (AP) or blast crisis (BC) chronic myeloid leukemia (CML) patients (pts) is unknown. The longest available follow-ups range from 2 to 3 years, with overall survival (OS) rates variable from 10 to 40% for AP pts and from 7 to 10% for BC pts. The objective of the study is to report the 5 to 6 year experience of the GIMEMA Working Party on CML (trial STI571/003) with IM 600 mg daily in 203 pts in AP or BC. Methods. Pts were monitored for hematologic response every month and for conventional cytogenetic every 6-12 months. Results. 203 pts were treated with IM 600 daily: 111 (55%) were in AP and 92 in BC (72 in myeloid BC and 20 in lymphoid BC). Overall, 155 (76%) are dead (including 156 in BC and 6 after allogeneic transplant) after 1 to 64 mos from start of IM (median: 24 mos). Median follow-up is 66 mos (55-73). 189 pts (93%) were previously treated for CML in chronic phase (CP) for 3 to 26 mos (median: 6), while 14 had the disease onset in AP/BC. Of 111 pts treated in AP, 106 pts (96%) showed a return to CP and 74 pts (70%) obtained also a complete hematologic response, which lasted at least 4 weeks in 68 (61%) pts. A complete cytogenetic response (CCgR) was achieved by 15 (14%) pts, in 2 to 15 mos (median: 4), with a major CgR rate of 25%. CCgR was subsequently lost by 8 pts after 3 to 50 mos from its first achievement, with a median CCgR duration of 21 mos. Overall survival decreased from 86% to 71% and 49% at 6, 12, and 36 mos, but remained stable thereafter (37% at 66 mos). The median survival time was 36 mos. Of 92 pts treated in BC, 57 pts (62%) returned to CP and 24 pts (26%) obtained also a complete hematologic response, which lasted at least 4 weeks in 22 pts. A complete cytogenetic response (CCgR) was achieved by 8 (9%) pts, in 2 to 11 mos (median: 4), with a major CgR rate of 20%. CCgR was subsequently lost by all but 2 pts after 2 to 12 mos from its first achievement, with a median CCgR duration of 7 mos. The median survival time for BC pts was 7 mos, with 8% of pts alive at 66 mos. Conclusions. IM 600 mg has a substantial activity when used as a single agent in pts with CML in AP and BC. Pts treated in AP showed a significantly higher hematologic and cytogenetic response rate, as well as better OS than BC pts. Overall, OS remains stable after 36 mos of therapy, indicating that IM therapeutic effect is durable over time.
EFFICIENT CYTOTOXIC ACTIVITY OF ZOLEDRONATE-ACTIVATED GAMMA DELTA T CELLS AGAINST IMATINIB-RESISTANT CML CELL LINES.

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Aminophosphonates activate human Vγ9Vδ2 T lymphocytes and promote their cytotoxic properties. We investigated the effects of activated gamma delta T cells on Ph+ leukemia cell lines, sensitive or resistant to imatinib mesylate. Gamma delta T cells were expanded from normal PBMCs by culturing with zoledronate 5 nM (Zometa, Novartis) and IL-2. The cytotoxicity of activated gamma delta T cells against CML cell lines was evaluated by flow cytometry based on double labelling with CFSE and PI. Gamma delta T cells were cytotoxic against all tested imatinib-sensitive (K562S) and -resistant (K562R, KCL22R, LAMA84R) cell lines, in dose-dependent manner. In addition, zoledronate treatment enhanced the sensitization of tumor cells to gamma delta T cell cytotoxicity resulting in good increased lysis. We next analyzed which subset was more effective in inducing lysis. Therefore, we separated FAC-sorter the four subsets of naïve, CM, EM and TEMRA, and tested the individual cytotoxic properties of each subset. We observed that the TEMRA subset had the higher cytotoxic activity. To determine potential mechanisms of gamma delta cytotoxicity against CML cells, we first explored the possible contribution of TRAIL. To this aim, we investigated whether TRAIL could induce cell death in CML cells cultured in presence of 100 ng/ml IL-2-TRAIL. All tested cell lines were sensitive to TRAIL-induced apoptosis; interestingly, whereas K562S cells were moderately sensitive to apoptosis, K562R and KCL22R were highly sensitive. Thus, we next analyzed the expression of TRAIL receptors, to understand the observed differences in TRAIL-sensitivity. We found that all tested cell lines expressed the death-inducing receptors DR4 (but not DR5), and that the K562R, KCL22R expressed higher levels of DR4, compared to K562S. In addition, all cell lines expressed weak levels of DcR1 and DcR2, which are thought to act as decoy receptors and they may desensitize the HMGR that blocks the IPP production, did not affect tumor cell lysis. Finally, NKG2D appeared to play a smaller role in cytotoxicity as anti-NKG2D antibody blocked cytotoxicity with a weak inhibition On the basis of obtained results we hypothesize that administration of Vγ9Vδ2 T lymphocytes and zoledronate may significantly induce antitumor activities in CML patients resistant to imatinib treatment.

Table.

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Gamma delta T cells was incubated in a flow cytometric assay based on double labeling with CFSE and PI. With CML cell lines at indicated E:T ratio.

PO-344
IMATINIB HIGH DOSE (800 MG) IN INTERMEDIATE SOKAL RISK CML PATIENTS IN CHRONIC PHASE: RESULTS OF A PHASE II TRIAL OF THE GIMEMA CML WORKING PARTY

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Background. Imatinib has become the treatment of choice for CML. The standard dose (SD) for chronic phase (CP) CML is 400 mg daily, results are less favourable in pts at high or intermediate Sokal risk vs low Sokal risk ones. In intermediate Sokal risk patients, the IRIS trial (Hughes et al NEJM 349:15, 2003) reported at 12 mos a complete cytogenetic response (CCGR- 0% Ph-pos) rate of 67% and a major molecular response (MMoR) rate of 45%. Pre-clinical and clinical data suggest that high doses (HD - 800 mg daily) of imatinib may be more effective. Aims. The GIMEMA CML Working party is conducting a phase II, multi-institutional prospective study (n. CML/021) to investigate the effects of imatinib HD in intermediate Sokal risk. Between Jan , 2004 and May, 2005, 25 centers enrolled 78 pts; median age 56 yrs (26-79) (24% were aged 65 years or more at enrollment). The median observation time is 18 mos. Results. At 6 mos, 81% obtained a CCGR and 54% of CCGR pts a MMoR (Bcr-Abl/Abl x 100 ratio < 0.05%). At 12 mos, the CCGR rate was 88% and the MMoR rate was 56%. Two patients progressed to accelerated/blastic phase. The compliance to HD treatment was good: at 3, 6 and 12 mos 56%, 53% and 54% of the pts received a median daily dose of imatinib equal or superior to 600 mg. Non hematopoietic AEs accounted for the great majority of dose reductions. Conclusions. The results of this trial further indicate that imatinib HD induces higher and more rapid responses in intermediate Sokal risk CML pts in early chronic phase, being superior to the results obtained with SD (IRIS) and in the range of the MD Anderson results (Kantarjian et al Blood 2004 103:2875).

A New class of dual SRC/ABL kinase inhibitors for the treatment of IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIAS

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Overcoming resistance to Imatinib remains a major challenge for successful treatment of Chronic Myeloid Leukemia. Imatinib resistance in vivo and in vitro is mainly mediated by mutations within the Bcr-Abl kinase domain, amplification of the Bcr-Abl or aberrant signals downstream of Bcr-Abl such as Src kinases and downstream RAS signalling pathways. Due to the high homology of Src and Abl kinases in their active conformation, Src inhibitors may be of additional therapeutic value for preventing or targeting drug resistance. Among them, Dasatinib was shown to be 325-fold more potent than Imatinib against wild-type Bcr-Abl and able to target most Imatinib-resistant Bcr-Abl mutations due to its conformation-tolerant binding to Abl kinase domain. Here we report a preclinical study on two pyrazolo[3,4-d]pyrimidine derivatives 4-amino-substituted, (S13 and S29) belonging to a new class of dual Src-Abl inhibitors. The results of enzymatic cell-free assays proved that S13 and S29 inhibit non-receptor tyrosine kinase activities, in particular Abl and Src, at IC50 doses ranging from 0.8 and 0.08 µM. We tried the cytotoxic effects of both drugs on Bcr-Abl-transduced cell lines (32D and Ba/F3) either sensitive or resistant to Imatinib. Imatinib resistance was provided by mutations at functionally distinct regions of the Bcr-Abl kinase domain, including Y253F and E255K and spontaneous mutations. Both drugs significantly reduced proliferation in semisolid assay of cell clones expressing the wild type and mutated Bcr-Abl genes, including T315I. LD50 ranged between 1.0 and 1.6 µM in all cases. Moreover, they were highly effective against cells rendered Imatinib-resistant by IL-3 and those transducing the p185 Bcr-Abl protein, usually referred to as controls for Imatinib-resistance (LD50 ranging between 0.8 and 0.7 µM).

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PO-346
POST-TRANSITIONAL MODIFICATIONS OF FOXO3A TRANSCRIPTION FACTOR ASSOCIATED WITH THE CONSTITUTIVE TYROSINE KINASE ACTIVITY OF P210 BCR-ABL PROTEIN. IMPLICATIONS FOR NEW TARGETED THERAPIES FOR CHRONIC MYELOID LEUKEMIA

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FOXO transcription factors are components of a highly conserved signalling pathway connecting growth and stress signals to transcriptional control. Central to their regulated activities is a shuttling system, which confines FOXO proteins to either the nucleus or the cytoplasm through post-translational modifications (phosphorylation and acetylation) and association with the nuclear transport machinery. Previous studies underscored the critical role of FOXO3a inactivation by Bcr-Abl in the maintenance of a leukemic phenotype, proceeding from its influence of proliferation, survival, glucose metabolism and response to stress. However, the mechanisms involved in p210 Bcr-Abl protein tyrosine kinase (TK) interactions with FOXO3a are still unknown. Results of the study presented here show that in murine myeloid progenitors (32D) stably transduced with Bcr-Abl construct the significant decrease of FOXO3a expression and phosphorylation in the cytoplasm in response to Imatinib (IM) parallels FOXO3a nuclear import and transcriptional induction of growth arrest (p27 and Gadd45) and pro-apoptotic (FasL and Bim) signals. Multiple events are involved in FOXO3a restored function. They encompass protein kinase B (Akt) dephosphorylation and inactivation and c-Jun NH2 terminal kinase (JNK) phosphorylation and activation resulting, in turn, in 14-3-3 phosphorylation and client protein release from cytoplasmic ligand. Interestingly, FOXO3a dephosphorylation was a protein event persisting up to 8 th hr of exposure to IM. Multiple events contributed to the protein re-phosphorylation in the nuclear compartment (precluding its nuclear export) by 24 th hr. They include the compensatory activation of p38 and p42/44 MAP kinases (that directly phosphorylate FOXO3a proteins at MAP kinase consensus sites) and the protein acetylation by p300 acetyltransferase (that weakens FOXO DNA-binding and resistance to Akt-mediated phosphorylation). Those findings may help to design new therapeutic strategies including drugs such MAP kinase or acetyltransferase inhibitors that may complement IM in the cure of CML.

PO-347
IMATINIB AND HYPERCHOLESTEROLEMIA

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We have previously described a rapid reduction of plasma total cholesterol level (PTCL) in a small series of patients, within one month after imatinib therapy (400 mg daily) was started for either chronic myeloid leukaemia (CML, 17 patients) or hypereosinophilic syndrome (HES, 2 patients). Here we report the same analysis extended over 19 patients. PTCL was evaluated at diagnosis and after one month of imatinib therapy (400 mg daily). Exclusion criteria were clinical or pharmacological conditions known to affect lipid metabolism, such as diabetes, alcoholism, oral contraception, assumption of drugs lowering cholesterol levels and modification of diet, weight or physical activity, before and in course of therapy. At diagnosis, 9 patients were hypercholesterolemic. Among them (mean PTCL, 254 mg/dL; range, 223-295) eight normalized their cholesterol levels (mean PTCL, 176 mg/dL; range, 160-187). In addition, among the normolipidemic patients, in 7 out of 10 cases, a less marked reduction of PTCL was observed (mean PTCL, 167 to 139 mg/dL; range, 145-185 to 112-163). A slight increase of PTCL was observed in 1 hypercholesterolemic (250 to 260 mg/dL) and 3 normocholesterolemic patients (mean PTCL, 163 to 180 mg/dL; range, 144-187 to 148-217). The ability of imatinib to lower circulating levels of cholesterol was persistent and long-lasting (median follow-up, 28 months, range 5-46). All patients achieved at least the complete haematological remission after one month of imatinib therapy. Imatinib inhibits several tyrosine kinases such as Abl, c-Kit and platelet-derived growth factor receptor (PDGFR). PDGFR-dependent signaling pathways have been shown to play a role in several non-malignant diseases including atherosclerosis, nephropathy and type 2 diabetes. Moreover, PDGFR binds specifically to and phosphorylates the cytoplasmic tail of low-density lipoprotein (LDL)-receptor-related protein (LRP), a member of LDL-receptor superfamily, which is known to act in areas as diverse as cellular signalling, degradation of proteases, liver cholesterol uptake and glucose-induced insulin secretion. Our observation suggests that in vivo modulation of the phosphorylation pathway may elicit a cholesterol-lowering activity. In the context of CML, these results are particularly impressive considering that an inverse correlation between PTCL and total leucocyte count has been clearly documented.

PO-348
CONJUNCTIVAL HEMORRHAGIC EVENTS ASSOCIATED WITH IMATINIB

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Abstract. Imatinib mesylate (formerly STI571; Glivec™, Novartis Pharm) is a selective inhibitor of Bcr-Abl, c-Kit, Arg and platelet-derived growth factor receptor (PDGFR) tyrosine kinases; it is a phenylamino-pyrimidine derivate that has the specific property of binding to the ATP docking site of the P210 oncoprotein, thus preventing self-phosphorylation and other consequent effects. Imatinib mesylate offers a new form of targeted therapy for the treatment of patients with chronic myelogenous leukaemia (CML) and gastrointestinal stromal tumours (GISTs). It is generally well tolerated and leads to no higher incidence of hemorrhagic events than other therapies. The study population included 90 consecutive patients (56 males and 34 females, M/F ratio 1.6; median age 53 years, range: 16-77) diagnosed as having CML between 1992 and 2006, who were followed up for a median of 52 months (range: 6-221) at the Hematology-Bone Marrow Transplantation Unit, Maggiore Hospital, Milan. All of them were treated with modulated doses of imatinib mesylate in accordance with the standard guidelines; 87 patients were treated with imatinib mesylate for a minimum of three months, ten of whom (11%) developed unilateral or bilateral conjunctival hemorrhage (CH): seven male and three females (M/F ratio 2.3) with a median age of 64 years (range: 23-77) and a median follow-up of 30 months (range: 8-182). They all had a Karforsky performance scale (KPS) of 100%, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0. All were in a chronic phase (nine complete and one partial cytogenetic remission). One subject presented a concomitant cutaneous hematomata, and two a previous episode of epistaxis and cutaneous hematomata. During the follow-up, no other hemorrhagic events were observed except for the recurrence of CH in six cases (7%). No other side effects of imatinib mesylate were observed, except for two episodes of generalised erythematous maculopapular and pruritic skin rash. No comorbidity was documented that could be considered as having a clear role in the etiology of CH. The CH spontaneously resolved within 14 days. As we did not observe any evident cause for such a high incidence of CH, we hypothesise drug hypersensitivity or a still unknown ocular cause possibly related to local irritation induced by imatinib mesylate.

PO-349
DELETIONS OF THE DERIVATIVE CHROMOSOME 9 DO NOT INFLUENCE RESPONSE TO IMATINIB IN EARLY CHRONIC PHASE CHRONIC MYELOID LEUKEMIA PATIENTS: A GIMENA CML WP ANALYSIS

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Background. Extensive submicroscopic deletions adjacent to the break-
point on derivative chromosome 9 (der[9]) have been reported in a subset of Chronic Myeloid Leukemia (CML) patients and have been associated with an adverse outcome with conventional drugs and α-interferon (±-IFN). Hunty et al. (Blood. 2003; 102:2205-12) reported 275 CML pts who were treated with imatinib in CP, suggesting that der(9) deletions were associated with lower response rates and a shorter time to progression. Different data were reported by Quintas-Cardama et al (Blood. 2005; 105:2281-6), who did not find any difference related with der(9) deletions in other 320 patients treated with imatinib. In these 2 studies, some patients began imatinib in early CP (51 and 152, respectively) while many patients (224 and 168, respectively) were treated in late CP. Aim To establish the relationship of der(9) deletions with the response to imatinib in early CP patients, we performed a sub-analysis within 5 simultaneously running trials of the GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Adulto) CML WF (n=CML/012, phase II - ima 500 in intermediate Sokal risk; CML/022, phase II - ima 400 vs 800 mg in high Sokal risk, n = CML/023, observational - ima 400 mg). Patients and Methods. 442 evaluable CML patients in early CP have been enrolled from January, 2004 to January, 2006. At enrollment, 55 (12%) of them had der(9) deletion and 387 (88%) had not. The 2 groups, with/without deletions, were comparable (no significant difference in age, Sokal risk, imatinib dose). Median observation time is 18 months (3-53 months). Fluorescence in situ hybridization (FISH) analysis of bone marrow cells was performed with 3-color dual-fusion probes. Response monitoring was based on conventional cytogenetic examination after 6 and 12 months on imatinib (every 6 months thereafter) and quantitative molecular (Q-PCR) evaluations (PB) after 3, 6 and 12 months on imatinib (every 6 months thereafter). Results. At 6 months, complete cytogenetic response (CCgR) rates were (deletions present/absent) 73%/67%, with a major molecular response (MMR, defined as a Bcr-Abl/ABL x 100 ratio < 0.05%) rate of 44%/45%, respectively. At 12 months, CCgR rates were 85%/84% and MMR 58%/57%. No difference is statistically significant. During the first year, no progression was reported among patients with deletions, while 6 (1.5%) of non deleted patient progressed to accelerated/blastic phase. Summary and Conclusions. The presence of der (9) deletions do not constitute a poor prognostic factor for response in early CP patients under imatinib treatment. Cytogenetic and molecular responses were similar in the 2 groups, with and without der(9) deletions, are superimposable. This finding is relevant to the long term effect of imatinib treatment, since both the CCgR and the MMoR are important and established indicator of long term survival. Acknowledgments. COFIN 2003, FIRB 2001, AIRC, CNR, Fondazione del Monte di Bologna e Ravenna, European LeukemiaNet, ALL.

**PO-350**

**RAPID RESPONSE TO DASATINIB IN A CML BLAST CRISIS PATIENT THAT SUBSEQUENTLY DEVELOPED SEQUENTIAL BCR-ABL MUTATIONS CAUSING MULTIDRUG-RESISTANCE: A CASE REPORT**

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Clinical resistance to Imatinib Mesylate (IM) has emerged as a major key in the treatment of Chronic Myeloid Leukemia (CML). Although higher doses of IM can overcome resistance in selected patients, alternative BCR-ABL inhibitors have been developed to suppress the activity of IM-resistant BCR-ABL mutants. Among them, Dasatinib, a dual SRC- and ABL-kinase inhibitor, has shown promising activity in CML patients unresponsive to IM. Here we report the case of a CML patient that firstly had an impressive clinical response to Dasatinib and then developed resistance because of sequential mutations in the same leukemic clone. A 63 year-old female was diagnosed with chronic phase CML in February 2006 and was started on IM 400 mg daily. She achieved a complete hematological response (CHR) within two months of IM-therapy with a decrease of her BCR-ABL/ABL ratio to 0.02912. However, in September 2006, she lost her CHR presenting with peripheral pancytopenia and the presence of immature myeloid circulating cells compatible with a progression to the AP of the disease. Her BCR-ABL/ABL ratio increased to 0.47899. She therefore commenced higher doses (600-800 mg daily) obtaining only a partial hematological response with no variation in the levels of her BCR-ABL transcript. In the following weeks, the patient developed an overt lymphoid BP and the E255K BCR-ABL mutant was documented in 85% of her leukemic clones. Bone marrow cytogenetic analysis showed 100% Ph+ cells with no evidence of other abnormalities. The patient received two cycles of standard induction chemotherapy (DNR and VCR) and was subsequently placed on Dasatinib therapy (70 mg bid) in January 2007. At the time, she displayed severe leukocytosis (WBC-count: 259.050/mm3) that responded dramatically to five days of Dasatinib (WBC-count: 1.290/mm3). Dasatinib therapy eventually led to severe pancytopenia and the drug was first reduced (70 mg daily) and then stopped. After the patient received supportive transfusional therapy, she re-started full dose Dasatinib promptly achieving a CHR. Bone marrow evaluation after two months revealed a major cytogenetic response (20% Ph+ cells) with a BCR-ABL/ABL ratio of 0.02052, that further decreased to 0.0023. At that point, PCR-based clonal DNA sequencing no longer detected the presence of the E255K mutant. Unfortunately, after a total of four months of Dasatinib treatment, the patient displayed disease progression (loss of CHR). RT-PCR analysis detected a marked increase of the BCR-ABL transcript (BCR-ABL/ABL ratio: 0.44174) and in the in vitro screening identified the coexistence of the E255K and the T315I mutants in 50% of the leukemic clones. Dasatinib therapy was discontinued and the patient died of uncontrollable disease. Recent evidence suggests that Dasatinib can induce hematological and cytogenetic responses in AP/BC CML patients unresponsive to IM. BCR-ABL in the patient reported here, Dasatinib rapidly induced both a CHR and, differently from these reports, a MCyR in a patient with the E255K mutant. However, despite this excellent initial response, the sequential selection of E255K and T315I mutations in the same cellular background strongly argues for major alterations in the DNA replication and repair mechanisms of this leukemic clone. Hence, our observations suggest that mechanisms causing genetic instability rather than BCR-ABL per se should represent the target of future experimental approaches for the treatment of advanced stage CML.**
PO-352
IMATINIB MESYLATED AND CARDIAC FUNCTION

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A few studies have appeared suggesting that Imatinib Mesylate (IM) may have cardiotoxic effects, which may lead to heart failure through decreased ejection function (EF), increased the left ventricular diameter and left ventricular disfunction. We investigate by echography a group of CML patients in long term treatment with Imatinib, with no pause in their treatment and with no known previous cardiac problems. Thirty patients with median age of 57 years, 17 men and 18 women, under treatment with IM for at least 4 years at full dose or with highest doses entered the study. EF was 52-73% (n.v. >55%), no patients had increased left ventricular diameter or left ventricular disfunction, and all echographic parameters were normal for the age. More than half of patients (16/30) showed low/intermediate grade valvular insufficiency, which can be considered usual for the age. Only a single patient (5.3%) had serious cardiac problems: a left atrial low dilatation, moderate tricuspid insufficiency, related to right atrial dilatation with low pulmonary hypertension and pulmonary valvular insufficiency and postero-lateral pericardial detachment in particular in right regions with a principle of diastolic collapse in lateral side of the right atrium, with normal systolic function of left ventricle. This patient was treated with highest dose of IM (800 mg/die) for one year. Thus, in our series IM-related cardiotoxicity is a relative rare event, unrelated to the dose and the length of treatment. Maybe preexisting cardiovascular problems, which may have remained undiagnosed are the basis for cardiotoxicity of IM; indeed, in a cohort of IM treated patients developing congestive heart failure (Kerkela et al, Nat. Med) 7/10 patients had hypertension, 4/10 were diabetic and 4/10 have history of coronary artery disease. Thus an ecocardiographic study should be performed at start of IM treatment and repeated during the follow-up especially when the first examination shows abnormalities. It could be useful to perform a case control study with a long term follow-up to clarify the real impact of IM on heart failure.

PO-354
GENETIC POLYMORPHISMS IN THE METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE ASSOCIATED WITH CHRONIC MYELOID LEUKEMIA (CML)

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Introduction. Folate metabolism plays an important role in carcinogenesis through DNA methylation and nucleotide synthesis affecting gene expression and DNA instability. Methylenetetrahydrofolate reductase (MTHFR) regulates intracellular folate metabolism and two common MTHFR single nucleotide polymorphisms (SNPs), C677T located in exon 4, and A1298C located in exon 7, have been reported to be associated with reduced enzymatic activity. Perturbation of folate metabolism as consequence of reduced MTHFR activity results in DNA hypomethylation and promotes DNA damage, through uracil misincorporation into DNA during replication, leading to an increased risk of DNA double-strand breaks during DNA excision repair and subsequent genetic instability. So far, conflicting results have been reported on the possible role of MTHFR polymorphisms and leukemogenesis and few studies have addressed this issue in chronic myeloid leukemia (CML). In this study a group of 109 patients with CML and a group of 270 racially matched healthy controls were genotyped for the two common MTHFR SNPs and overall frequencies were compared. Methods. DNA was extracted from bone marrow or peripheral blood samples. PCR-RFLP assays were used to assess the two common polymorphisms in the MTHFR gene. Results: MTHFR 677T genotype frequencies in CML and controls were 25.29% and 13.76%, respectively. MTHFR 1298C genotype frequencies in CML and controls were 11.76% and 7.84%, respectively. The MTHFR 677T genotype frequency in CML patients was significantly different from controls [p=0.03]. MTHFR 1298C genotype frequency was only marginally lower in cases compared to controls [p=0.08]. Discussion. On the basis of this results it is suggested that decreased MTHFR activity associated with the presence of the 677T genotype may result in increased stability of DNA leading to a protective effect against CML. Also the effect of the 1298C genotype, even less prominent, on DNA stability cannot be excluded. Understanding genetic susceptibility to CML, with particular respect to folate metabolism, will allow the identification of novel therapeutic strategies. Further studies are ongoing in a larger population of CML patients, also in relation to potential susceptibility to ABL kinase domain mutations development and subsequent resistance to therapy with tyrosine kinase inhibitors. Results will be presented. Acknowledgments. Supported by: European LeukemiaNet, COFIN 2003, AIL, AIRC, Fondazione del Monte di Bologna e Ravenna.

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Hypcholesterolemic effect of imatinib in patients affected by chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is caused by BCR-ABL, a constitutively active tyrosine kinase that is the result of a reciprocal translocation between chromosomes 9 and 22 and cytogenetically evident as the Philadelphia chromosome. Identification of the BCR-ABL kinase fusion protein and its central role in the pathogenesis of CML provided new opportunities to develop rational molecular targeted therapies. Imatinib, a specific and potent inhibitor of BCR-ABL, has become the standard drug therapy for CML, and has dramatically diminished the use of allelo-potent stem cell transplantation. In our institution we are following 20 patients with CML (12 F and 8 M, median age: 45 years, r: 19-66 years). All patients, at diagnosis, underwent a treatment with imatinib (400 mg/day). The therapy was well tolerated with minimal collateral effects. After one year from the start of treatment 16 out of 20 patients achieved complete molecular remission of disease, while four patients with a sub optimal molecular response received a dose escalation of imatinib (600 mg and/or 800 mg/day). Actually all patients are alive: 17 out of 20 show a complete molecular remission, while three patients show a sub optimal molecular response. Interestingly, by analysing the routine biohumoral parameters we have found in four patients with initial high cholesterol levels (up to 300 mg/dL) a significative decrease of them with a normalization of serum cholesterol levels after six-months from the beginning of imatinib administration. All four patients did not use any hypcholesterolemic drug. Although there are several published data on the metabolic effects of imatinib administration little is still known about the effects of imatinib on lipid pathways, such as cholesterol metabolism and in particular: cholesterol de novo biosynthesis, uptake of exogenous cholesterol LDL receptor mediated, cholesterol esterification, cholesterol efflux HDL receptor mediated. Moreover, in our hands this imatinib metabolic effect seems to be limited only to patients with previously high cholesterol levels. The significance of that and the clinical relevance of serum cholesterol level decrease in a long-term therapy, such as imatinib administration, are still unknown.

Ocular Side Effects in Chronic Myeloid Leukemia Patients Treated with Imatinib


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Ocular serious side effects in imatinib treated patients are rarely reported. We describe our experience on ocular toxicities occurred in imatinib treated CML patients, which consisted in a wide spectrum of effects ranging from peribital oedema to serious adverse events (glaucoma). From January 2000 to September 2006 we treated with Imatinib 250 Ph+ CML patients; overall we recorded ocular toxicity in 10.4% of the patients, of which 14 were serious events. Mild peribital oedema (grade I) is a common effect caused by Imatinib. We reordered this complication in 70% of the 250 patients, with no treatment required. Conversely, in those patients (30%) with moderate and severe peribital oedema (grade II and III) diuretic treatment was necessary, in particular when toxicity was visually disabling. Four patients had increased intraocular pressure, which in one case was associated to abnormal persistent vision and in another case was associated to recurrent conjunctival haemorrhage (two patients had as predisposing factor myopia and 3 patients were treated with high dose imatinib); two patients, one affected by diabetes and one by hypertension, experienced glaucoma, which in one instance was complicated by recurrent vitreous haemorrhage. Both discontinued imatinib: one patient was treated surgically and one with acetazolamide, and ocular defects being resolved with imatinib gradually restarted. Two patients had recurrent retinic haemorrhages: both had predisposing factors (one patient myopia and one hypertension); the side effect occurred early (1-2 months). Four patients experienced recurrent conjunctival haemorrhages with 2 of them having pre-existing myopia and 1 hypertension; 2 patients had received 800 mg/day and 2 patients 400 mg/day. Median time of ocular toxicities occurrence was 15.5 months (range 3-24). One patient experienced an optical neuritis after 51 months of treatment with imatinib at standard dose; this side effect required drug discontinuation for 6 weeks and resolved with vitamine therapy and oral steroids. We observed several ocular side effects that could likely be considered as related to imatinib therapy (possible events). We noticed that these complications preferentially occurred in patients treated with high dosages (600-800 mg/day in 7/13 patients); conversely, in all patients in whom ocular effects occurred under at 400 mg/day, we recorded the presence of predisposing factors consisting in hypertension and myopia.

Development of Chronic Myeloid Leukemia in a Patient with Multiple Myeloma: A Case Report

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Reports of both multiple myeloma (MM) and chronic myeloid leukaemia (CML) occurring in the same patient are extremely rare. The present case report documents such an occurrence in a patient affected by MM who developed CML after an interval of 7 years. A 58 year old female was diagnosed with Stage IA (Durie Salmon) MM IgG lambda in December 1998 and stage IIIA in October 2008. The patient was treated with melphalan at a dose of 8 mg/m2 days 1-4 for 8 courses and zoledronic acid for 26 courses. In January 2005 developed osteonecrosis of the jaw and chemotherapy was interrupted. In December 2005, 7 years after MM diagnosis, the patient developed a CML. Peripheral blood showed progressive leukocytosis, white cell 20000 mm3 (90% neutrophils, 2% metamyelocytes, 1% myelocytes, 5% lymphocytes, 2% monocytes), hematocrit 33%, haemoglobin 11.4 g/dl, platelet 88000 mm3. A bone marrow biopsy with aspiration revealed a predominance of mature and intermedia forms of granulocytes consistent with the diagnosis of CML. Cyrogentic analysis revealed the presence of the Philadelphia chromosome (Ph1) chromosome-46, XX, t(9;22) CML. Peripheral B/ABL mRNA transcripts were detected with reverse transcription polymerase chain reaction. The patient received imatinib mesylate (400 mg once daily) and after 3 months the values of peripheral blood were within normal limits and monoclonal component was stable. In April 2007, 9 years after MM diagnosis and 16 months after CML diagnosis, the patient is alive and with good performance status. The simultaneous occurrence of MM and CML or development of MM in patients with CML or of CML in patients with MM, are extremely rare events. Were reported (Medline research) 6 cases of concurrent MM and CML (Lewis, Br J Haematol, 1986; Ritzmann, Am J Hem, 1996; Esposito D, Del Avina E, 1972; Boots, J Clin Pathol, 1982; Tanaka, Acta Hematol, 1998, Schwarzmeier, Leukemia, 2003), 3 cases of MM in patients with CML (Darghazanian, Can Med Assoc J, 1974; Zouboms, Br J Haematol, 1967; Vener C, J, 2003) and 4 cases of CML in patients with MM (Klenn, Yearse Med J, 1993; Paitta, Am J Hema- tol, 1985; Dumouchel, Sem Hop, 1983; Nitta, Int J Hematol, 1999). Also there are several reported cases of MM occurring with other myeloproiferative disorders. The literature dates support the existence of a relationship between MM an CML, suggesting that the two neoplasms arise from transformation of a common pluripotent stem cell.

Low dose dasatinib in refractory imatinib mesylate chronic myeloid leukemia: case report

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Imatinib mesylate, an inhibitor of BCR/ABL tyrosine kinase, has radically changed chronic myeloid leukemia (CML) treatment; however, if well tolerated in most patients, some of them could be refractory and develop resistance or intolerance. Dasatinib is a new, orally bioavailable ABL kinase inhibitor that is two log more potent than imatinib and active against 14 of the 15 imatinib-resistant BCR/ABL mutants. We here describe the case of a 58 year-old woman that was diagnosed as having...
high Sokal and Euro score risk CML Ph+ in March 2002. After debulking with hydroxyurea, she started imatinib mesylate therapy at the daily dosage of 400 mg. One month later the patient obtained a complete hematological remission, but at the first bone marrow analysis performed on July 2002 we observed only a minor cytogenetic response (MCyR, 21/50 Ph+ metaphases, 42%). Unfortunately, the following evaluations documented the lost of MCyR (10/2002 and 04/2003). Furthermore, the treatment had been complicated by hematological toxicity, in particular severe neutropenia and thrombocytopenia (grade IV sec. WHO) even during imatinib dose reduction (500 mg daily, 05/2003). In May 2004, considering the lack of response and therapy toxicity, we started IFN-alpha at the daily maximum tolerated dosage of 5 MUI. This drug had been frequently interrupted because of the development of relevant hematological toxicity and none cytogenetic response. After the exclusion of punchform BCR/ABL mutations, we began dasatinib (70 mg bid): the therapy had been characterized by severe neutropenia which caused frequently drug withdrawal and dosage reduction up to 20 mg bid (03/2006). Despite this low dosage, in November 2006, for the first time she obtained a partial cytogenetic response (42/50 Ph+ metaphases, 84%), confirmed at the last evaluation performed in April 2007 (55/58 Ph+ metaphases, 94.8%). During the follow-up she didn’t discontinue low dasatinib dosage showing a very good quality of life and drug tolerance, with complete and stable normalisation of hematological parameters and gaining a major cytogenetic response.

**PO-359**

**EFFICACY OF DASATINIB IN TWO PATIENTS WITH IMATINIB-RESISTANT CHRONIC MYELOID LEUKEMIA AND ELEVATED THROMBOCYTOSIS**


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Dasatinib is a novel multi-targeted oral inhibitor of bcr/abl, a constitutively active tyrosine kinase that causes chronic myeloid leukaemia (CML). Dasatinib has been recently approved by the FDA and EMEA for use in Philadelphia-positive leukemias in patients who are resistant or intolerant to Imatinib. We report here two Imatinib-resistant CML patients with progressive disease and high platelet count, in whom Dasatinib was highly effective in inducing complete hematologic remission. The first patient was a 57-year old female, with a diagnosis of Ph+ CML performed on December, 2001. She also had a thyroid adenoma, treated with surgery and L-tiroxine. The patient initially received alpha-Interferon (9,000,000 U i.w) up to April 2002, when she started therapy with Imatinib 400 mg/d. After 18 months of Imatinib treatment (December, 2003), despite a good hematological response, her karyotype analysis revealed the persistence of 8/20 abnormal Ph+ metaphases. Imatinib was then increased up to 600-800 mg/d, obtaining a partial cytogenetic response. However, on October, 2006, an increase in platelet count was observed (PLT 900×10³/mm). The patient initially received alpha-interferon at the daily maximum tolerated dosage of 5 MUI. This drug had been frequently interrupted because of the development of relevant hematological toxicity and none cytogenetic response. After the exclusion of punchform BCR/ABL mutations, we began dasatinib (70 mg bid): the therapy had been characterized by severe neutropenia which caused frequently drug withdrawal and dosage reduction up to 20 mg bid (03/2006). Despite this low dosage, in November 2006, for the first time she obtained a partial cytogenetic response (42/50 Ph+ metaphases, 84%), confirmed at the last evaluation performed in April 2007 (55/58 Ph+ metaphases, 94.8%). During the follow-up she didn’t discontinue low dasatinib dosage showing a very good quality of life and drug tolerance, with complete and stable normalisation of hematological parameters and gaining a major cytogenetic response.
**PO-361**

**NON-PEGYLATED LYPOSOMAL DOXORUBICIN, CYCLOPHOSPHAMIDE, VINCRISTINE, PREDNISONE AND RITUXIMAB (R-COMP) AS INITIAL TREATMENT FOR PATIENTS WITH SPLENIC MARGINAL ZONE LYMPHOMA (SMZL): A GISL STUDY.**


Background. SMZL is an indolent lymphoma, presenting with massive splenomegaly generally associated with intrasinusaloid bone marrow infiltration. The encapsulation of doxorubicin into non-pegylated liposomes allows targeting of the drug to affected organs including spleen, lymphnodes and bone marrow. Methods. In 2005 the GISL started a phase II study for the treatment of patients with histologically confirmed SMZL, investigating safety and clinical profile of 6 courses of a modified R-CHOP regimen in which standard doxorubicin was substituted with non pegylated lyposomal doxorubicin (NPLD) used at the same doses (50 mg/m²) (R-COMP). Main inclusion criteria were age > 18 yrs, normal cardiac function and active disease (at least one of the following: Hb <10/g/dl; plt <100.000/mm²; symptomatic splenomegaly, elevated LDH, B symptoms, extrasplicnic disease, LDT <12 months). Splenectomy was allowed prior to treatment start only in case of symptomatic spleen enlargement. The study was planned according to a two-stage Simon design using overall response rate as primary endpoint. We present the results of the analysis performed after the completion of study stage 1. The access to stage 2 is allowed if at least 12 responses are recorded among among the first 19 evaluable cases. Results. As of April 2007, 20 patient were enrolled with the following characteristics; median age 63 years (80-80), Hb <10/g/dl in 20%, Lymphocytes > 5000/mm² in 65%, elevated LDH in 65%; 2 patients were splenectomized before treatment start. One patient was not available for response assessment. A clinical response was observed in all remaining 19 cases (ORR 100%); 12 (63%) cases obtained a CR. So far only one patient progressed at +1 month from treatment. Treatment was well tolerated with grade III/IV neutropenia in 8 patients (42%), grade III pulmonary toxicity in 1 patient and grade II peripheral neuropathy in 5 cases. In one case a reversible cardiac failure was reported after 1 month from the end of treatment. Conclusions. In conclusion 6 cycles of R-COMP combination represent a safe and promising treatment option for patients with clinically active SMZL.

**PO-362**

**MJMA CHEMOTHERAPY: A VERY EFFECTIVE SALVAGE REGIMEN FOR NON-HODGKIN’S LYMPHOMAS AND A STRONG MOBILIZER OF PERIPHERAL BLOOD STEM CELLS (PBSC).**

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Background. The prognosis of patients with aggressive non-Hodgkin’s lymphomas who fail to achieve complete remission on frontline chemotherapy, or relapse early thereafter, is severe. In such cases bone marrow transplantation after major debulking with conventional chemotherapy is indicated. So there is clinical interest in regimens that can couple both efficacy against lymphoma and activity in PBSC mobilization. Materials and Methods. A pilot study was conducted with a regimen, MJMA, including mitoxantrone 10 mg/sqm iv day 1, carboplatin 200 mg/sqm iv day 1-2, methylprednisolone 500 mg/sqm iv day 1-3, and cytarabine 2000 mg/sqm iv day 3, for 6 cycles every 21 days. Sixteen patients were treated with the following histology: 7 follicular, 6 large B-cell, 1 mantle-cell, 1 anaplastic large-cell, and 1 peripheral T-cell lymphoma. Median age was 45 years (range 25 to 81). Thirteen patients had stage IV (all with bone marrow involvement) and 3 stage III disease. Preceding lines of chemotherapy were 1 in 6 cases, 2 in 2, 3 in 7 and 4 in 1. Ten presented refractory disease, 6 were in relapse. Results. Response was complete in 10 patients, partial in 5 and null in 1. The only remarkable toxicity was hematological, since all the patients required support with G-CSF and 4 had grade 3-4 thrombocytopenia. In 8 patients, candidate to high-dose chemotherapy program, an adequate number of CD34+ PBSC was mobilized and harvested (from 4.06 to 22.8x10¹⁰/Kg, median 12.2x10¹⁰/Kg). Two subjects out of these 8 did not actually undergo the planned transplantation, responded completely and are still well without further therapy after 80 (follicular lymphoma, refractory) and 73 months (large B-cell lymphoma, refractory), respectively. The 6 transplanted cases were completely debulked before transplantation and were conditioned with melphalan 200 mg/sqm + mitoxantrone 80 mg/sqm. Five of them are still without evidence of disease at 6, 14+, 18+, 51+, 103+ months, one relapsed after 12 months. Out of the 8 patients not candidate to transplantation, 2 had complete remission (8 and 15 months long, respectively), 5 responded partially and 1, with an anaplastic lymphoma for which MJMA was the fifth-line therapy, did not respond and died three months later. Conclusions. Though tested in a severe clinical setting, MJMA is a relatively very effective salvage chemotherapy. Moreover, it has a high activity in PBSC mobilization, allowing adequate harvest of CD34+ cells.
PO-364  
R-COMP VS R-CHOP IN THE TREATMENT IN NEWLY DIAGNOSED ELDERLY PATIENTS WITH AGGRESSIVE NON-HODGKIN’S LYMPHOMA
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Introduction. Preclinical studies evidenced that liposomal anthracyclines preserve antitumor activity but reduce the incidence and the severity of cumulative dose-related cardiomyopathy. In this study we evaluated the impact of R-COMP (containing non-pegylated liposomal doxorubicin - Myocet) as compared to R-CHOP regimen (containing conventional doxorubicin) as a first line therapy in newly diagnosed elderly patients with aggressive NHL. Methods. In the last two years, 40 pts with aggressive NHL were enrolled in the study. Twenty pts: M/F 11/9, median age 69 years (range 57-81) received R-COMP treatment in stage III-IV, IPI score: intermediate grade 13 pts, high grade 7 pts. Baseline median left ventricular ejection fraction (LVEF) was 58% (range 50-68). R-COMP (with liposomal doxorubicin 50 milligrams/mq on day 1) regimen was administered every 21 days for six cycles. Twenty pts: M/F 9/12, median age 66 years (range 57-70) received R-CHOP treatment in stage III-IV, IPI score: intermediate grade 14 patients, high grade 6 pts. Baseline median LVEF was 62% (range 55-70). The CHO regimen (with conventional doxorubicin 50 milligrams/mq on day 1) was repeated every 21 days for six cycles. Results. Arm R-COMP: Out of 20 pts, 15 (75%) achieved CR, 4 (20%) PR and 1 (5%) did not respond. After a median follow up of 13 months, all 20 (100%) pts resulted alive. At the end of treatment, LVEF median was 57.90%. We did not observe cardiac events related to therapy. The toxicity was mild with grade 3 neutropenia in 4 (20%) pts. Arm R-CHOP: 14 pts (70%) achieved CR, 4 (20%) PR, and 2 (10%) were refractory. Out of the 14 patients in CR: 1 died for infection and 1 relapsed after 10 months. After a median follow up of 13 months, 19 (95%) pts resulted alive and 12 (60%) of which in continiue CR. Grade 3 neutropenia was observed in 4 (25%) pts. At the end of treatment, LVEF median was 62%. Conclusions. In our hands the substitution of conventional with liposomal doxorubicin into the R-CHOP regimen at an equivalent dose has been a feasible, active and well tolerated choice in elderly pts with newly diagnosed aggressive NHL. Long-term follow up will be required in order to verify the outcome of DLBCL pts with reduced LVEF.

PO-365  
FLUDARABINE PLUS CYCLOPHOSPHAMIDE (FC) IN PATIENTS WITH PREVIOUSLY UNTREATED LOW-GRADE LYMPHOMA
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Introduction. Fludarabine alone or in combination with other drugs has been recently reported to be effective in the treatment of previously treated low-grade Non-Hodgkin’s Lymphomas (LG-NHL). The aim of this work was to define the therapeutic efficacy and toxicity of a combination of fludarabine and cyclophosphamide (FC regimen) in untreated LG-NHL. Methods. In the last 4 years, forty-five untreated patients with LG-NHL, were enrolled in our study. All pts (M/F: 28/17, median age 62 years) were treated with three-day courses of fludarabine 25 milligrams/mq/day, cyclophosphamide 300 milligrams/mq/day, every four weeks for a maximum of six courses. G-CSF, when necessary, and Pneumocystis Carinii prophylaxis was given. Among 45 pts, 21 (46%) received diagnosis of small lymphocytic, 9 (20%) of mantle cell, 8 (18%) of follicular grade I and 7 (16%) of immunocytoma. Results. Out of the 45 pts, 43 (94%) achieved complete response (CR), 1 (3%) partial response, while the remaining 1 (2%) showed no benefit from the treatment. In the follicular and mantle cell subtype we observed a CR rate of 100%. Median duration of follow-up was more than 27 months. Overall Survival (OS) and Disease-Free Survival (DFS) rates were 93% and 88%. Hematologic grade 3-4 toxicity was seen in only five (11%) pts; no opportunistic infections or deaths were associated with the administration of the FC regimen. Discussion. Our data show that the FC combination chemotherapy for untreated pts with LG-NHL has a significant level of activity with prolonged CR. In addition the schedule is well tolerated and without significant toxicity. A prolonged follow-up will be needed to determine the long-term efficacy of this treatment regimen.

PO-366  
THERAPEUTIC ROLE OF RITUXIMAB IN THE TREATMENT OF INTRAVASCULAR LARGE B-CELL LYMPHOMA
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Background. Intravascular large B-cell lymphoma (IVL) is a rare form of non-Hodgkin lymphoma, characterized by the growth of large neoplastic lymphocytes within blood vessels. Diagnosis is often difficult and outcome generally poor. The standard therapy remains to be defined; anthracycline-based chemotherapy is the most commonly used treatment. A few recent case reports seem to suggest a positive impact of rituximab on outcome of rituximab-containing regimens. Aim: to explore activity and efficacy of rituximab (375 mg/m² on day 1) plus CHOP or CHOP-like chemotherapy (R-CT) in comparison to the same chemotherapy alone (CT) in CD20-positive IVL patients selected from the largest series collected in Western Countries [Ferretti AJM, et al. Haematologica, 2007]. Methods. 27 IVL patients eligible for anthracycline-containing regimens were evaluated. Clinical features and outcome of 7 patients treated with R-CT strategy were compared to a group of 20 patients treated with CT. Results. Median age of patients was 67 yrs (range 39-86 yrs). Fourteen patients were males. The most commonly involved organs were: skin (12 patients), CNS (7), bone marrow (8), spleen (5), and liver (4). B symptoms were complained by 20 (74%) patients and an elevation of serum LDH was present in 21/24 (88%). Anemia, leukenopia and thrombocytopenia were observed in 20/26 (77%), 8/26 (30%), and 9/26 (35%) cases, respectively. Nineteen (73%) patients presented with stage IV disease. No significant differences in patients' characteristics between R-CT and CT subgroups were observed. After a median follow-up of 26 months, 15 patients achieved complete remission (CR), and 5 partial response (PR) (R-CT is still ongoing in 2 PR patients); 6 patients experimented progressive disease, and one patient (CT subgroup) died of toxicity. All the R-CT patients (5) who completed the treatment (100%) and 10 (50%) CT patients achieved CR (p<0.06). The only variable related to CR rate was the addition of rituximab. All lymphoma-related events in CT patients were observed within the first year of follow-up; 6 CT patients are alive and disease-free at a median follow-up of 71 months. Conversely, all the 7 R-CT patients are alive and relapse-free at a median follow-up of 16 months. The 2-year EFS was 85% in CT group and 100% in R-CT group (p<0.0001). The 2-year OS was 45% and 100%, respectively for CT and R-CT (p<0.0001). Conclusions: this international study suggests that, like for other CD20-positive lymphomas, the addition of rituximab to anthracycline-based chemotherapy could significantly improve outcome in IVL, a rare malignancy where prospective trials are lacking so far. Confirmatory analysis after a longer follow-up is warranted.
PO-367  
**EXCELLENT PROGNOSIS AND PREVALENCE OF HCV INFECTION OF PRIMARY HEPATIC AND SPLENIC NON HODGKIN’S LYMPHOMA**

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Background. Primary Hepatic (PHL) and Primary Splenic (PSL) non Hodgkin’s Lymphoma are rare entities. Small series of PHL and PSL have been reported, suggesting a non fortuitous association with Hepatitis C Virus (HCV) infection. It is believed that the prognosis is dismal, leading to early recurrence and short survival. Patients. We retrospectively reviewed PHL and PSL patients diagnosed in our institution between 1990 and 2005. Results. Twenty-five adult patients were identified, 6 with PHL and 19 with PSL. Twenty-four patients had a B-cell lymphoma, defined as Diffuse Large B Cell Lymphoma in 18. The prevalence of HCV infection was 68% in PSL and 66% in PHL. Combination chemotherapy was the mainstay of treatment; all PSL, but one underwent splenectomy before chemotherapy. Complete remission was achieved in all cases after front line therapy and only a few relapsed and responded to additional chemotherapy courses. Most patients presented with aggressive histologic subtypes, 92% are alive with a median follow up of 79 months. HCV infection did not appear to influence the results of therapy. Conclusions. Our study confirms the rarity of PHL and PSL and shows a high prevalence of HCV infection and demonstrates that the outcome of patients with PHL and PSL seems to be favourable.

PO-368  
**HIGH DOSE SEQUENTIAL CHEMOTHERAPY: AN EFFECTIVE TREATMENT IN RELAPSED AND REFRACTIVE HODGKIN’S LYMPHOMA**

Dept of Hematology, Careggi Hospital and University of Florence, Italy

The optimal salvage treatment for primary refractory and relapsing patients is still not defined. High-dose chemotherapy followed by autologous stem cell rescue proved to be more effective than conventional schemes. The German Hodgkin Study Group obtained good results with high-dose sequential chemotherapy (HDST) and autologous stem cell transplantation (ASCT) in this subset of pts. Since March 2002 to July 2006 16 pts were treated with HDST in our institution. The first phase was constituted by 2 cycles of DHAP; the second phase by cyclophosphamide 4g/sqm, methotrexate 8 g/sqm, etoposide 2g/sqm every 14 days; the third phase was ASCT with BEAM conditioning. The first and second phase were delivered on outpatient basis. After the first phase a CT evaluation was performed to assess the chemosensitivity; pts not achieving at least a partial response (PR) according to Cheson were considered off-study. Leukapheresis was performed after high-dose cyclophosphamide, and G-CSF 5 microg/Kg was administered from day +5 until collection. Pts status at the enrolment were: 7 relapsed within an year after the obtainment of CR; 7 refractory to the ABVD induction therapy ABVD; 2 refractory to conventional salvage treatment for relapse occurred after 9 years. Patients characteristics at diagnosis were: median age 28 years (range 19-42); 8 pts were female; 7 had B symptoms; ECOG performance status were 0 in 10 pts, 1 in five, 2 in one. All pts received the two cycles of DHAP without delays. After this phase 7 pts were excluded, 5 for stable/progressive disease, 2 for not achieving a PR (the late relapsed pts, who are still alive with disease). Nine pts completed the therapy according to the schedule, and after the ASCT all of them were in complete remission (CR) with negative PET scans. A CR patient developed a myelodysplastic syndrome 15 months after the ASCT and died from acute graft versus host disease; a patient relapsed after 139 days, and died of progressive disease. The other seven pts are still alive and in CR. Median time to treatment failure for the nine pts who completed the HDST was 31 months (range 139 days-52 months). HDST is an effective therapy for relapsed and refractory Hodgkin’s lymphoma. A better outcome was observed in the relapsed pts. After more than 30 months of median observation we observed a case of myelotoxicity, but a longer follow-up could better evaluate the real toxicity of this treatment.

PO-369  
**THERAPY OF PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS: A FIFTEEN YEAR EXPERIENCE AT FLORENCE UNIVERSITY HOSPITAL**

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Primary central nervous system lymphoma (PCNSL) is a rare tumor, accounting for approximately 1% of all intracranial neoplasms. Standard regimens for systemic lymphomas are ineffective in PCNSL, mostly because of the blood-brain barrier. High-dose methotrexate-based (HD-MTX) regimens represent the current treatment approach, in association or not with whole-brain radiation therapy (WBRT), which alone results in high response rates but frequent relapse. Since December 1991 to January 2005 25 patients (pts) with proven diagnosis of PCNSL were treated at Hematology and Radiotherapy Units of Careggi Hospital. Pts were treated with WBRT alone, with HD-MTX regimens alone or with a combined WBRT-HD-MTX therapy (CMT); these different approaches reflect the evolution of guideline in the therapy of PCNSL. Thirteen (52%) pts were male, the median age at diagnosis was 62 years (range 20-73 years). PCNSL was localized in the cerebral lobes in 17 pts (two with multiple sites) and in deep structures in eight pts (4 in cerebellum, 2 in corpus callosum, 2 in basal ganglia). Raised intracranial pressure was present in eight pts, eight had cognitive deficits, three had visual field defects, two had hypostension, two had seizures. Twelve pts were treated with WBRT alone, seven with HD-MTX regimens alone and six with a CMT. At the end of therapy 19 pts were in CR (76%) and two obtained a partial remission (8%), with an overall response rate of 84%. Four pts progressed during therapy. The CR rate was 66% for those treated with WBRT alone, 86% in pts treated with HD-MTX regimens alone and 83% in those who underwent CMT; these differences were not statistically significant. After a median follow up of 33 months (range 2-105 months) 18 pts died (sixteen due to disease) and 7 are alive and disease-free. The actuarial overall survival (OS) was 42% at 3 years and 27% at 5 years. PFS at 5 years was 22%. The presence of multiple lesions and the newly developed Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic model (based on age and performance status) were significant in the multivariate analysis for OS. In conclusion, we observed a higher (but not statistically significant) rate of CR in pts treated with chemotherapy (either alone or in combination with radiotherapy) over those treated with radiotherapy alone, whilst a difference in OS was not evident. MSKCC prognostic model and presence of multiple lesions showed significance in multivariate analysis for OS.
**PO-370**

BORTEZOMIB (VELCADE) COMBINED TO INVOLVED FIELD RADIOTHERAPY (IFR) IN REFRACTORY/RELAPESE HODGKIN LYMPHOMA (HL) AS SALVAGE THERAPY: REPORT OF TWO CASES

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Bortezomib has been successfully used to treat patients with refractory/relapase Multiple Mieloma (MM) and Non Hodgkin Lymphoma (NHL) with overall response rate of 85% and 58% respectively, whereas few studies have tested the efficacy and safety of Bortezomib as 2nd line therapy in HL patients (Younes et al., 2006). We have started a salvage treatment including Bortezomib combined with Involved Field Radiotherapy (IFR), in refractory relapsed HL to test the safety of such treatment. From September 2006 to March 2007, we have enrolled in this study two young patients with relapse and refractory HL respectively. Both patients have previously received 2 chemotherapy lines including Autologous Peripheral Blood Stem Cell Transplant (AUTO PBSCT). Case 1. A 16 yrs old girl in Partial Response (PR) after AUTO PBSCT five yrs before. The treatment scheme was: Bortezomib 1.3 mg/m² i.v. on days 1,4,8,11 of a 21 day cycle, for a total of 6 cycles. The response of evaluation was done after the 6th cycle of Bortezomib treatment (total dosage 30-50 GY). The role of cytology to diagnose and classify non-Hodgkin’s lymphoma (NHL) is controversial. In this study we have evaluated the diagnostic role of the combined application of fine needle cytology (FNC) and flow cytometric immunophenotyping (FCI). For this purpose, we have retrospectively evaluated 30 patients (mean age 54 years; range 16-82 years; 17 male and 13 female) seen at our Institutions in the last two years with a suspected diagnosis of lymphoma. In 26 cases the patients presented with palpable masses (site: 7 cervical, 2 supraclavicular, 1 inguinal, 10 axillary, 1 breast, 1 palatine tonsil, 2 paraortic) and FNC was done without any radiological guidance. In the remaining 4 cases FNC was performed on deep-seated masses (2 abdominal and 2 mediastinum) under ultrasound and CT guidance. In all but three cases (two reactive hyperplasias and one lymphoblastic T-cell lymphoma needing immediate therapeutic intervention for mediastinum involvement) node biopsy was subsequently performed. FNC yielded adequate material in all cases. Smears were stained with Diff Quik and Papanicolaou stain in each case. Material was also collected in transport buffer for flow cytometry. A large panel of directly conjugated monoclonal antibodies was used: CD45, CD2, CD3, CD5, CD4, CD8, CD20, CD11, CD20, CD22, CD28, kappa and lambda. In selected cases immunocytochemistry was performed aiming to evaluate the reactivity of CD45, CD30, CD15, EMA, cyclin D1, ALK-1 antibodies. The diagnostic categorization of cases on the basis of smear examination and FCI is shown in Table 1.

**Table 1. Diagnosis made by FNC and FCI combin analysis.**

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodgkin’s disease</td>
<td>6</td>
</tr>
<tr>
<td>Small lymphocytic lymphoma</td>
<td>5</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>5</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>2</td>
</tr>
<tr>
<td>T-cell lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Burkitt type lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>Anaplastic T-cell lymphoma</td>
<td>1</td>
</tr>
<tr>
<td>T-cell lymphoma of HAM/Ts origin</td>
<td>1</td>
</tr>
<tr>
<td>Reactive hyperplasia</td>
<td>2</td>
</tr>
</tbody>
</table>

**PO-371**

BENDAMUSTINE PLUS RITUXIMAB IS AN EFFECTIVE AND WELL TOLERATED TREATMENT FOR RELAPSED LOW-GRADE NON HODGKIN’S LYMPHOMAS AND CHRONIC LYMPHOCYTIC LEUKEMIA


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**Introduction.** Low grade non Hodgkin’s lymphoma (NHL) and chronic lymphocytic leukemia (CLL) are indolent malignancies characterized by indolent natural history and the inability to achieve cure through conventional therapeutic approaches. Monoclonal antibodies represent a good therapeutic option for this disease and have been shown in vitro to be active against cell lines that are resistant to other alkylating agents. In this study, we evaluated the efficacy of Bendamustine and Rituximab in patients with relapsed non Hodgkin’s lymphoma and chronic lymphocytic leukemia. **Methods.** From January 2006 until present we treated in our Haematology Unit five patients affected by the following diseases: 3 CLL and 2 indolent NHL (follicular and lymphoplasmacytoid histologic subtypes). Median age of patients was 65 years (range 60-71) and the median number of previous treatment was 3 (range 2-5). The treatment consisted of: Rituximab 375 mg/m² on day 1 and Bendamustine on day 2 and 3 (90 mg/m² for NHL and 70 mg/m² for CLL). This association was repeated every 28 days for 4-6 cycles. **Results.** All patients completed the treatment without relevant toxicity with a median follow-up of 4 months (range 1-11). Three of five patients responded to therapy, corresponding to an overall response rate of 60%. One patient affected by CLL obtained a complete remission (CR), the other two patients affected by NHL obtained uncompleted CR (uCR) and a partial remission (PR), with histologic subtype characterized by follicular and lymphoplasmacytoid type respectively. The two non-responder patients were affected by CLL (one of which carrying 17p deletion). Myelosuppression and thrombocytopenia were rare. **Conclusions.** The association of Bendamustine and Rituximab offers the potential for an effective new regimen without the toxicity associated with other chemoimmunotherapy regimens. The combination of Bendamustine and Rituximab therefore seems a feasible and efficacious regimen associated with very low toxicity for pluriresistant patients affected by non Hodgkin’s lymphomas and chronic lymphocytic leukemia and furthermore represent a good salvage therapy for patients relapsed or not eligible for other aggressive options because of age or comorbidity.
The systemic use of high-dose Methotrexate for newly diagnosed Primary Central Nervous System Lymphoma (PCNSL) has improved the median overall survival from 12 to 44.5 months. However, salvage treatment will be necessary in patients with refractory disease. 

**PO-373**

**SAVAGE SALVAGE FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA WITH **Y-^{90}** IBRITUMOMAB AND TEMOZOLOMIDE**

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The systemic use of high-dose Methotrexate for newly diagnosed Primary Central Nervous System Lymphoma (PCNSL) has improved the median overall survival from 12 to 44.5 months. However, salvage treatment will be necessary in patients with refractory disease. **Y-^{90}** IBritumomab Tiuxetan (Zevalin) is a murine anti-CD20 covalently linked to the high-energy beta emitter Yttrium-90 by the chelator Tiuxetan. Although mabs are large molecules, they could leak across permeable tumor vasculature into CNS lymphomas. Material and Methods. To evaluate this hypothesis, two patients with relapsed large B cell positive PCNSL, were scheduled to receive Rituximab on day 1 (250 mg/m²), 111In – Ibritumomab for imaging and **Y-^{90}**Ibritumomab on day 8 (0.4 mCi/Kg). Thereafter their treatment included a total of up to 8 maintenance cycles of Temozolomide (200 mg/m² on days 1-5). Results Case 1. 51-year old female, first diagnosed in August, 2002, after treatment with MTX plus ARA-C she obtained a complete remission until November 2005 when for relapse Rituximab, **111**In- Ibritumomab and **90**Y-Ibritumomab (0.4 mCi/Kg) were administered. Reevaluation six weeks after treatment showed a complete resolution of the tumor. After 10 months this patient maintained CR. Case 2. 68 year old female, first diagnosed in July 2003, after MTX and RT obtained complete remission until September 2005, when for relapse Rituximab, **111**In-Ibritumomab and **90**Y-Ibritumomab (0.4 mCi/Kg) were administered. Reevaluation six weeks after treatment demonstrated a complete disappearance of all enhancing abnormalities on Gadolinium-enhanced MRI. Conclusions. Even if Monoclonal Antibodies are large molecules, their favourable pharmacodynamic properties may overcome the pharmacokinetic limitations because based on their high specificity targeting lymphoma cells they do not necessitate a high concentration in CNS to induce a response in PCNSL as reported anecdotally after systemic Rituximab. The observed response to treatment is encouraging and compares favourably with other salvage regimens.

**PO-374**

**HIGH MOLECULAR REMISSION RATE IN HIGH RISK FOLLICULAR LYMPHOMA WITH SEQUENTIAL HIGH-DOSE THERAPY AND RITUXIMAB AS PURGING IN VIVO**

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We investigated a novel therapy protocol and more aggressive approach to high risk CD20 Follicular Centre Lymphoma (FCCL) to be implemented as a salvage regime after relapse or in case of resistant or as an up-front regime. The treatment included a combination of chemotherapy, specific immunotherapy and high dose therapy sequentially given. A first phase consisted of 4 CHOP courses, I and III phases were devoted to mobilise CD34 positive cells with high-dose Cytoxan and Cytarabine + G-CSF, Rituximab as purging agent in vivo and the final phase included the administration of BEAM scheme as conditioning regimen to autologous reinfusion of CD34+ cells. Clinical immunophenotypic and molecular parameters were used for evaluation of response. Up to July 2005 42 patients- mean age 56.6 years- completed this management protocol and analysed in this study. All patients had marrow involvement and were considered at high risk either because of grade 3 histology (14 pts) or IPI score > 2 (17 pts) or progression after previous treatment (11 pts) or B symptoms (8 pts). After CHOP therapy 17 patients obtained Complete Clinical Response (CCR), all but one had residual disease detected by immunophenotype in the marrow, none obtained a molecular remission. Following high-dose Cytoxan as mobilizing treatment and Rituximab as purging agent, 25 patients (60%) developed CCR, 21 pts Complete Immunophenotypic Remission (CIR) and 9 pts (21%) Complete Molecular Remission (CMR). Following high-dose Cytarabine and second course Rituximab, 34 pts (81%) reached a CCR, 29 had a CIR and 19 (47%) a CMR in the marrow sample. At the end of the entire treatment and following PBSC 59 pts (94%) reached CCR; one patient died of sepsis during cytopenic period, 36 pts (88%) resulted in CIR and 30 (71%) pts in CMR. At 39 months – mean- follow-up 36 pts (86%) remained in CCR and 29 (69%) still in CMR. Among the 3 patients relapsed, 2 had a residual molecular disease at the end of the treatment. One patient died of brain hemorrhage 5 months after the end of the treatment. In conclusion this study demonstrated that sequential therapy including purging in vivo with Rituximab and high-dose therapy is highly effective in inducing durable CR in most of FCCLs.

**PO-375**

**RITUXIMAB DOES NOT INCREASE TOXICITY OF CHEMOTHERAPY IN HCV+ AGGRESSIVE NON HODGKIN LYMPHOMAS**

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Combined chemotherapy with anti-CD20 monoclonal antibody (rituximab) is the most effective and safe treatment for de novo aggressive non Hodgkin Lymphomas (NHL). Some reports described increased hepatic toxicity-related incidence in HCV+ NHL, with infectious complications. South of Italy is a region with a high prevalence of HCV infection among the population. In our study we examined 20 consecutive HCV+ patients affected from aggressive non Hodgkin’s Lymphoma. Characteristics of the patients at diagnosis were: median age 65 years, Ann Arbor Stage III/IV. All pts were affected with diffuse Large B Cell Lymphoma. Liver biopsy, EGDS, hepatic function parameters, HIV, HBV and HCV markers were evaluated in all cases. We treated all patients with R-CHOP scheme at standard doses (R: 375 mg/m²). Antiviral treatment was indicated in no patient before starting the treatment. No patient was HbsAg positive. Four patients presented with elevated (ALT) alanine-aminotransferase (5x) before starting the therapy, in 16 patients hepatic function was within normal limits. During the treatment we observed progressive ALT level rise (10x) in 3/20 patients; in the remaining 17 patients (one with increased ALT level at diagnosis) no significant modification in the ALT levels was observed, neither during the course of treatment, nor in the follow up. The three patients with raised ALT before treatment were submitted to antiviral treatment (interferon-alfa) and recovered normal hepatic function with negative viral load after two months from the stop of the therapy; 6/20 patients who were HBcAb positive did not show viral reactivation during the treatment. Up to March 2007 all patients were in good general conditions, in complete remission of the disease, but one, who died after 2 cycles of chemotherapy because of myocardial infarction. Efficacy and safety of the treatment was evaluated in the following 6-12 months after the diagnosis. Thus, chemotherapy plus rituximab is well tolerated even in HCV+ patients and the clinical follow up is necessary during the course of treatment because hepatic toxicity may occur in 15% of patients. Compared to the great efficacy of the combined therapy with rituximab, the temporary hepatic toxicity, probable in a small proportion of patients and characterized by a relatively rapid recovery (2 months) confirms that R-CHOP is the gold standard even for NHL patients infected by HCV.

**PO-376**

**DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) IN ELDERLY PATIENTS TREATED WITH D-VICEMB PROTOCOL IN THE PRE- RITUXIMAB ERA**

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We previously reported the results of D-VICEMB (Dexamethasone, Vinblatine, Cyclophosphamide, Etoposide, Mitoxantrone and Bleomycin) protocol in 30 untreated patients aged >70 years with intermediate-high grade non Hodgkin’s Lymphoma (NHL) (Haematologica haematologica/the hematology journal | 2007; 92(s3) | 189
2002;87:1227). As regards to DLBCL, 24 patients older than 70 years were treated with the D-VICEMB protocol from 1996 to 2002. Their median age was 72 years (range 70-85); 14 patients were female, 14 patients were symptomatic, 13 had extranodal disease, 11 had LDH value higher than normal, 4 had bulky disease and 13 had performance status (KPS) <80%. 12 patients were stage II and 12 were stage III-IV. According to the IPI score 1 patient was low risk, 8 patients were low-intermediate risk, 10 high-intermediate risk and 5 high risk. 16 patients (67%) achieved a complete remission (CR) and 6 (25%) obtained a partial response (PR), for an overall response rate (ORR) of 92%. With a median follow-up of 75 months (7-112), overall survival was 42%. Twelve patients are alive and twelve deceased (8 for lymphoma progression; 1 for bladder cancer; 1 for colon cancer; 1 for heart failure and 1 for sudden death). Only 3 relapse occurred among the 16 CR patients, but 2 patients dead for other neoplasms and 2 for comorbidities. Event free survival for this patient’s group was 47%. Increasing age has a negative impact on the outcome of patients with aggressive NHL. In scientific literature people aged 60 years and older treated with chemotherapy have a lower response rate than younger patients. In elderly patients, the treatment should be individualized. In our experience, the therapy for elderly patients affected by aggressive NHL but combinations of Rituximab plus other polichemotherapy regimens were not largely employed. Should be interesting to evaluate the role of Rituximab in association to weekly regimens in this group of patients.

**PO-377**

**A PILOT STUDY OF FCR (FLUDARABINE, CYCLOPHOSPHAMIDE, RITUXIMAB) FOLLOWED BY 90Y-IBRITUMOMAB TIUXETAN (ZEVALIN®) IN RELAPSED FOLLICULAR LYMPHOMA (FL) PATIENTS**


**Background.** FL is the second most frequent type of NHL and accounts for 25% of lymphoma cases, rituximab chemotherapy combinations have shown to increase significantly PFS. However the natural history of this lymphoma leads to a recurrence of the disease. After 1st relapse patients are considered good candidates for salvage chemotherapy: combinations regimens, myeloablative therapy. FCR regimen has provided encouraging results in FL and Zevalin has been reported to be effective in patients with relapsed or refractory FL. We are conducing a pilot study in relapsed FL to evaluate the efficacy and safety of FCR combination followed by Zevalin. **Methods.** At date reporting for this abstract we have recruited 4 patients median age 65 yr (range 57-77). Two patients (1 with stage IV and 1 with stage II) were previously treated with CHOP regimen and a cycle of RCHOP. In reality, the patient’s group was 47%. Increasing age has a negative impact on the outcome of patients with aggressive NHL. In scientific literature people aged 60 years and older treated with chemotherapy have a lower response rate than younger patients. In elderly patients, the treatment should be individualized. In our experience, the therapy for elderly patients affected by aggressive NHL but combinations of Rituximab plus other polichemotherapy regimens were not largely employed. Should be interesting to evaluate the role of Rituximab in association to weekly regimens in this group of patients.

**PO-379**

**NASAL TYPE LYMPHOMA: A RARE HEPATOSPLENIC RELAPSE**

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Nasal-type lymphoma is a rare peripheral T cell lymphoma, associated with poor prognosis despite aggressive chemo and local radiotherapy (Gallamini et al, Blood 2004;103: 2474-2479; Al akeem DA et al., Oral Oncol, 2006;Oct 23) Patients commonly presents with nasal symptoms: mass, obstruction or bleeding. This malignant T cell proliferation is characterized by an angiocentric grow pattern in sinusoids and large conflu-ent areas of necrosis. (Radiger T et al., Pathology 2006; Dec 29). The diagnosis is essentially based on the clinical presentation of extranodal ulcerative lesions in the upper aerodigestive tract and histopathologi-cal-immunohistochemical NK/T phenotype (CD2+/CD56+) may also involve skin, soft tissues and testis. (Radiger et al./Reinartz SM et al Eur Arch Otorhinolaryngol 2007, 264: 59-45; Falchook et al., abs 2460 J of Am soc of Haem Vol 108 n 171) 10-20% of patients have a history of immunodepression. (Liang R Cin Lymphoma 2000 Jun.) and a clonal Epstein-Barr virus (EBV) infection is typically found in lymphoma T cells (Radiger T et al). Treatment with standard anthracycline regimens has been to date disappointing, with variable response, high relapse rate and short median survival (37,9-43,5% in 5 years) (Al hakeem/Xiao...
Half of patients with Stage I disease and relapse disease is invariably fatal. Consolidation with high dose chemotherapy + autologous stem cell transplantation or dose dense chemotherapy (Megachop) may improve the outcome (Rodríguez et al. 2006 ASH I) (Kouzaky H et al. Auris Nasus Laryns 2006 Mar) and successes have been also reported with the use of CD52 monoclonal antibody (Gallamini). However, the optimal treatment has not been defined yet. Case Description. A previously healthy 57 years old lady presented with, nasal bleeding and obstruction due to a mass in the right nasal cavity expanding to maxillary bone sinus. Hystopathological analysis revealed T/NK nasal type lymphoma. Blood Sample was positive for EBV antibodies. TC, BM biopsy and MR evidenced early stage I E with ethmoidal nasal bone localisation. Six courses of CHOEP chemotherapy were given, followed by involved field RT (86 G). RC was obtained at intermediate restaging, but a new ischiatric bone lesion soon appeared, which was treated by RT. Although SCT from her HLA-compatible brother was offered but was refused by the patient. Sixth months later left maxillary erosion, gingival, cutaneous purple lesions, hepatic mass and anemia revealed relapse confirmed by MR, TC and gingival biopsy. High dose Ara C was refused by the patient; therefore, low dose VP16 + Dexametasonse were given, with very short lasting FR. The patient died two months later 15 months from diagnosis. Discussion. Our patient presented with typical nasal- maxillary lesion, whereas unusual localisations such as liver and ischiatic bone appeared at relapse. The outcome was unfavourable, in spite of early stage presentation, absence of underlying immunodeficiency and combined chemo-radiotherapy treatment, this confirming the poor prognosis of nasal-type NK/T lymphoma. Consolidation with autologous SCT appears to be the best therapy, since anecdotal long-term remissions have been obtained. In our case that procedure was repeat- edly refused by the patient.

PO-380
TREATMENT OF HIGH RISK DIFFUSE LARGE B-CELL LYMPHOMA WITH INTENSIFIED INDUCTION THERAPY AND HIGH DOSE SEQUENTIAL THERAPY
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Diffuse large B-cell lymphoma (DLBCL) represents 40% of all non-Hodgkin lymphomas (NHLs). The standard first-line treatment for DLBCL is CHOEP, but its results in high-risk patients (IPI age adjusted 2 or 3) is still not satisfactory. The use of high-dose therapy with autologous stem cell transplantation (ASCT) in first-line treatment led to discordant results. We treated young high-risk patients (pts) with DLBCL with an intensified induction therapy followed by HDST and ASCT. Pts with DLBCL, age less than 55 years, and low-intermediate IPI with bulky disease or high IPI were eligible for this study. Treatment consisted of three phases: the first phase was constituted by three cycles of an intensified CHOEP (cyclophosphamide 3000 mg/m2; doxorubicin 75mg/m2; vincristine 1,4 mg/m2 on day 1 and prednisone 100 mg for 5 days). Peripheral stem cells collection was performed after the third cycle. The second phase (HDST) consisted of cyclophosphamide 4g/m2 on day 1, methotrexate 8g/m2 on day 15 and etoposide 2g/m2 on day 29, all with G-CSF support. The third phase was HD-T-ASCT, with a condition of mobilized of melphalan 50 mg/m2 on day 1 and mitoxantrone 60 mg/m2 on day 1. Since March 2002 we enrolled 13 pts, with median age of 37 years (range 22-49). Eight had a stage III-IV (62%), 6 presented B-symptoms (46%), 11 had bulky disease (85%), 6 pts had more than one extranodal localisation (46%). Two pts had a WHO performance status 2 (15%), and 10 had elevated LDH (77%). Twelve pts completed the scheduled treatment, while 1 died during the first phase because of a sepsis. At the end of therapy 10 pts obtained a CR (83%), 2 pts were in partial remission (PR). After the first phase no pts have obtained a complete remission (CR), 23% of pts obtained a CR after the second phase and 10 patients (77%) have obtained the CR at the end of third phase. After a median follow-up of 27 months (range 7-52) progression free survival (DFS) was 79%, after a median observation of 42 months (range 2-55) 11 patients (82%) were alive; two pts died, one because of a sepsis and the other (a PR patient) for disease progression. We can conclude that this protocol represents an attempt to improve the results of HDST adding an intensified treatment in the first phase. This therapy was feasible and effective in a high risk group of pts. Rituximab could further improve these results and could also be used in pts with involvement of bone marrow at diagnosis.

PO-381
HODGKIN LYMPHOMA: AN UNUSUAL OCULAR LOCALIZATION?
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Introduction. Neurological manifestations in Hodgkin Lymphoma (HL) are rare; the reported incidence is 0.5%. They can be caused by an involvement of central nervous system (CNS), prevalently during relapsing disease and in immunodeficiencies, like AIDS. The spread of intracranial sites is prevalently vascular, while rarely an invasion from involved lymph nodes occurs. Methods. A 17-year-old girl experienced fever, headache, dizziness, diplopia, decreased vision and the concomitant presence of supracavicular lymphadenopathies; the histopathological examination was consistent with HL, nodular sclerosis. Bone marrow biopsy revealed negative and the CT scan showed mediastinal lymphadenopathies of 2.5 cm and a splenic lesion of 3 cm. The impairment of visual acuity, little scotomas and diplopia were associated to bilateral optic disc swelling, macular edema. CT and MRI of the brain were negative, cerebrospinal fluid normal, while EEG showed aspecific abnormalities. Results. Yet after the first polychemotherapeutic infusion according the COPP/ABV scheme a visual improvement occurred, with progressive rapid reduction and than disappearance of the papilledema and other pathological findings of the fundus. Finally complete remission occurred and the ocular manifestations of the disease resolved completely. After 6 chemotherapeutic cycles and radiotherapy, CT scan and 18FDG-PET resulted both negative, still lasting after 1 year. Discussion. Even if rare, an involvement of CNS in HL must be considered in the presence of neurological signs, in particular in immunocompromised and in progressive cases. At the time of this paper no literature has yet discussed and such a rapid response of the visual manifestations to systemic HL treatment seemed to suggest a direct CNS involvement, even if CT, MRI and cerebrospinal fluid exams didn’t. Moreover the papilledema is not specific and it could be caused even by inflammation. As well as a direct CNS involvement, neurologic signs in HL could rarely be due to more remote tumor effects in the form of paraneoplastic syndromes, whose pathogenesis isn’t understood. As regarding the effect of antiblastic therapy the important antiinflammatory effect of prednisolone of COPP could itself explain the regression of ocular paraneoplastic or inflammatory symptoms, while hematopoietic barrier could be difficulty passed by COPP/ABV chemotherapy drugs. A MRI of Optic Nerve probably could be useful in resolving the diagnostic question of our patient.

PO-382
TREATMENT OF HIGH-GRADE NON HODGKIN LYMPHOMA IN VERY ELDERLY PATIENTS
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Background. The incidence of non Hodgkin lymphoma (NHL) has increased over the past decade. The mean age at diagnosis is over 60 and therefore in the future NHL will be diagnosed more often in elderly people. Some studies have suggested that elderly patients with aggressive NHL are generally not treated in an optimal way in order to avoid toxicity, although this can reduce the likelihood of achieving a complete response. Methods. To evaluate our treatment modalities and results, medical records of all pts 75 years or older with newly diagnosed aggressive NHL referred to our Institutions for initial management were reviewed. Treatment was considered inadequate if deviating from standard NHL therapy. Results. 40 patients fulfilled the entry criteria, 37 (92.5%) patients were affected by Diffuse Large B Cell Lymphoma, 2 (5%) by Anaplastic Lymphoma and 1 (2.5%) by High Grade T cell Lymphoma. The median age was 79 (range 75-88). Of these patients 36

haematologica/the hematology journal | 2007; 92(s3) | 191
Standard chemo-immunotherapy is highly effective and generally feasible. However, only 24 of 32 patients (75%) had optimal number of cycles and dose, 8 patients (22%) received a suboptimal number of cycles (≤ 6). Thus resulting in a total of 24 pts treated as their younger counterpart, 12 patients treated with a suboptimal or not adequate treatment. The most important reason for sub optimally treated was poor PS and/or preexisting reduced cardiac function. Grade 3-4 toxicity was observed in 5 patients (20%) (4 hematologic toxicity and 1 non-hematologic toxicity). One patients died for cardio-toxicity in complete remission before completing the therapy. Among the 24 adequately treated patients, overall response rate was 91% (18 CR and 4 PR). 1 had stable or progressive disease and 1 died during chemotherapy. Overall 3-year survival among adequately treated patients was 60% and median survival time was 17 months. Two patients relapsed after 2 year of CR and 1 pts died in CR for myocardial infarction. Conclusions. Standard chemo-immunotherapy is highly effective and generally feasible in very elderly patients with aggressive NHL. If adequately treated, these patients have good chances to obtain a clinical response. Age alone should not be a contraindication to treatment.

### PO-383

**CENTRAL NERVOUS SYSTEM (CNS) PROPHYLAXIS IN ELDERLY PATIENTS WITH AGGRESSIVE B CELL- NON HODGKIN LYMPHOMA (NHL) AND ACUTE LEUKEMIA (AL): SAFETY AND EFFICACY OF INTRATHecal LIPOSOMAL CYTARABINE (ILC)**


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ILC is a sustained-release new cytarabine formulation that ensures sustained cytotoxic drug concentrations in cerebrospinal fluid; it has proven to be useful as intrathecal (IT) treatment of neoplastic and lymphomatous meningitis. Our aim was to verify, in elderly (>60 yrs) NHL and AL patients: a) the efficacy in preventing CNS lymphoma/leukemia progression, b) the feasibility and the toxicity of ILC treatment. From June 2005 to February 2007, 10 pts (median age 69 yrs, range 62-73), negative for the presence of lymphoma/leukemia cells in cerebrospinal fluid by cytology and flow cytometry, have been treated. Of these, 6 were classified as having stage IV IPI 3 DLBCL, 2 stage IV mantle cell NHL (MCL), 1 lymphoblastic and 1 undifferentiated acute leukemia (AUL). Extranodal diseases were present in 4/6 DLBCL. As first line systemic treatment, six NHLs were given R-CHOP, one CHOP and one MACOP-B regimen. The 2 AL pts received conventional three drug induction therapy (vincristine + idarubicine + prednisone) every week for 3 weeks, followed in post-CR phase by 3 courses of L-VAMP (vincristine + cisplatine + intermediate dose methotrexate + leucovorin rescue) and by conventional maintenance (6-mercaptopurine, methotrexate and monthly reinduction with vincristine + prednisone). ILC was given, in NHL pts, at dosage of 50 mg followed by systemic steroid injection, the day before chemotherapy for a total of 4 doses; in AL pts it was given every 4 weeks during induction and maintenance for a total of 6 doses. Up to April 2007, after a median follow up of 8 months (range 2-22) from the diagnosis, no isolated CNS relapses have been observed. ILC has been well tolerated; side effects (cephalea and neutropenia WHO I grade) being mild and manageable in all the patients. Seven pts (6 NHL and 1 ALL) achieved CR, 1 pt relapsed after 6 months from CR and died during II line therapy, 2 NHL pts are still in therapy. Due to its efficacy and ease of administration, ILC (DepoCyteR) is a convenient prophylactic drug mainly in elderly patients. These findings justify the development of clinical trials to evaluate the use of flow-cytometry and cytopsin techniques to identify at diagnosis the NHL patients at risk as well as the efficacy and safety of IT depot cytarabine to prevent NM involvement in ALL and NHL patients.

### PO-384

**PET-CT F18-FDG DIAGNOSTIC ACCURACY IN LYMPHOMA; OUR EXPERIENCE**

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Aim of the study: Use of positron emission tomography (PET) / computed tomography (CT) in Hodgkin Lymphoma (HL) and Non Hodgkin Lymphoma (LNH) continues to expand worldwide. F18-FDG PET-CT is currently widely utilized for response assessment after completion of therapy and for pretreatment staging and assessment of response during therapy (therapy monitoring). We were interested to evaluate the impact of this nuclear technique in staging and in the predictive value of F18-FDG PET-CT. Evaluation during and at the end of therapy. Materials and Methods. From October 2005 to March 2007 we studied forty-seven patients (21 female, 26 male; age range 21-79) with HL/NHL. In total we performed 64 scans; each patient fasted for 6 hours and then was injected with 4.0 MBq/kg body weight of F18-FDG. 60 minutes later image acquisition was performed using a dedicated PET/CT tomograph (Discovery LS-GE Medical System). 20 patients were studied in pre-treatment staging (8 Patients were newly diagnosed lymphoma) and 17 patients for post therapy response assessment (2 during therapy for early respond assessment). All patients underwent CT at staging and post-therapy; 32 patients received chemotherapy and radiotherapy, 15 patient only chemotherapy. Results. In 6 patient (75%) with newly diagnosed lymphoma PET-TC F18-FDG was substantially concordant with CT with an evaluation based on involved lymph-nodes regions or organ analysis, in 2 patients (25%) patients PET-CT was superior to CT showing other sites of disease. In 10 (50%) patients in pre-treatment staging, PET-CT showed more sites of disease than CT evaluation, with significant upstaging from stage II to stage IV. In 11 (23%) patients, the PET-CT post-treatment evaluation, modified the therapy planning (second line chemotherapy, radiotherapy etc.). Conclusions. In our preliminary data, FDG-18 PET-CT has shown to be a relevant non invasive method of staging of lymphoma and was superior compared with CT in nodal and extranodal detection of abdominal disease. Moreover scan results during therapy and at the end of the first line therapy seem to be a good predictor of outcome of the patient.
EFFECTIVE ANTI-TUMOR IMMUNOMODULATORY PROPERTIES OF ZOLEDRONIC ACID

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Introduction. Zoledronic acid (Zol) is the most potent aminobisphosphonate clinically available. Preclinical in vivo data suggest that it modulates the development of bone disease, decreases tumor burden with a direct anti-cancer activity, and reduces the migration and the metastatic invasion of cancer cells. Zol efficacy in anti-cancer adjuvant therapy also rests on its anti-angiogenic properties and ability to expand gamma/delta T cells, both in vitro and in vivo. The published data thus far available concern murine models of transplanted tumors. Present work was made to assess Zol immune-mediated activity in impairing spontaneous carcinogenesis in a transgenic mouse model.

Results. NeuT mice were treated with 16 administrations of 100 µg/Kg of Zol divided into four courses of a single weekly injections for four weeks followed by a three weeks rest. Zol administration was started when mice were 7 weeks old and therefore when all the 10 mammary glands display a widespread atypical hyperplasia. Zol was administered intravenously (i.v.) or into the mammary pad (i.mam.). Mice were evaluated for: 1) tumor onset, 2) tumor multiplicity and 3) overall survival. Results. Data obtained from these first experiments have shown that a similar significant tumor growth impairment was evident in mice receiving Zol administered i.v. or i.mam. Preliminary results have demonstrated Zol capacity to induce a significant increase in the percentage of gammadelta T cells in the spleen and in the lymphonode of Zol-treated mice. Even more importantly, data obtained in IFN gamma, which is a well known mediator of innate and adaptive immune responses. Zol immune-mediated anti-tumor properties are further and even more convincingly supported by the evidence that NeuT mice knocked-out for the gene encoding for the Fcy receptor (Fcγ-KO NeuT) and NeuT mice knocked-out for the gene encoding for the perforin (Prf-KO NeuT) did not benefit of Zol anti-tumor action. Conclusions. All together, these data show for the first time that Zol in vivo anti-tumor functions at least partially relies on broad immunomodulant properties, involving cellular and humoral immunity.

Figure. Zol treatment schedule. 100 µg/Kg of Zol were injected i.v. or i.mam. at the indicated time points (black arrows). The arrows shows when mice received 100 µg/Kg Zol i.v. or i.mam. Zol administration started when all the 10 mammary glands already display a widespread atypical hyperplasia.

PAIN IN PATIENTS WITH ADVANCED HAEMATOLOGICAL MALIGNANCIES FOLLOWED AT HOME

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Background. Although a high occurrence of pain in the haematological population has been claimed, no study on this topic concerning the Home Care (HC) setting has been reported. Aims. In order to address this issue, a prospective study in a haematological HC setting has been conducted. Methods. Pain syndromes presented by patients followed at home over a 6-years period was properly recorded and classified. Pain, as fifth vital sign, was assessed, during a clinical visit, every 24 hours until analgesia, and then every 3 days. The pain intensity was reported by a Numerical Analogue Scale (NRS), which rated 0 (no pain), 1 to 3 (mild pain), 4 to 6 (moderate), 7 to 10 (severe) or by a verbal description scale including four items: no pain, mild pain, moderate and severe pain. Pain management was based on the WHO analgesic ladder and included causal measures if applicable. Results. There were 258 (55%) males, median age was 67 (4-95) years and the median Karnofsky Performance Status was 50 (10-70). They were followed at home for a mean of 72+144 (range: 1-1132) days. Out of 469 patients, 244 (52%) experienced a total of 264 pain syndromes (Table 1), the intensity of which was rated from mild to moderate in 81% and from moderate to severe in 69% of them.

Table 1. Patient’s demographic features, incidence and distribution of pain syndromes according to the haematological diagnosis.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients</th>
<th>MF</th>
<th>Mean Age (years)</th>
<th>Incidence of pain</th>
<th>Pain syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>128</td>
<td>119</td>
<td>60 (15-91)</td>
<td>32 (82%)</td>
<td>52 (1-24)</td>
</tr>
<tr>
<td>ANLL</td>
<td>29</td>
<td>15</td>
<td>66 (13-97)</td>
<td>11 (38%)</td>
<td>7 (0-10)</td>
</tr>
<tr>
<td>MDS</td>
<td>52</td>
<td>44</td>
<td>65 (18-90)</td>
<td>26 (50%)</td>
<td>13 (1-18)</td>
</tr>
<tr>
<td>MDS</td>
<td>18</td>
<td>17</td>
<td>70 (19-92)</td>
<td>11 (61%)</td>
<td>7 (0-18)</td>
</tr>
<tr>
<td>CMPD</td>
<td>34</td>
<td>34</td>
<td>61 (19-88)</td>
<td>28 (82%)</td>
<td>17 (0-26)</td>
</tr>
<tr>
<td>MMM</td>
<td>15</td>
<td>11</td>
<td>65 (22-85)</td>
<td>5 (33%)</td>
<td>6 (0-10)</td>
</tr>
<tr>
<td>MM</td>
<td>5</td>
<td>5</td>
<td>65 (43)</td>
<td>5 (100%)</td>
<td>7 (0-15)</td>
</tr>
<tr>
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<td>5</td>
<td>65 (43)</td>
<td>5 (100%)</td>
<td>7 (0-15)</td>
</tr>
</tbody>
</table>

MM: Multiple Myeloma; ALL: Acute Lymphoblastic Leukemia; NHL: Non Hodgkin Lymphomas; BC: Blastic Crisis, HD: Hodgkin Disease; CLL: Chronic Lymphocytic Leukemia, Acute Myeloid Leukemia, MDS: Myelodysplastic Syndromes, CMPD: Chronic Myeloproliferative Disorders.

The diagnosed pain mechanism were: 56% deep somatic, 15% superficial somatic, 14% visceral, 8% mixed and 7% neuropathic of pain syndromes. Moreover, out of 284 pain syndromes, 150 (51%) were caused by bone involvement. Incident pain was observed in 38% of all pain syndromes. In all malignancies, deep somatic pain was prevalent. In addition, 85% of visceral pain syndromes were observed in non Hodgkin lymphoma patients. The most frequent pain causes were bone marrow expansion, osteolysis, lymph nodes enlargement and mucusitis. An effective control of pain at rest was attained in 259/284 (92%) of pain syndromes, although a completely stable pain relief was achieved in 202/284 (71%) of them, with a lower rate of response in the cases complicated by neuropathic and incidental features compared to continuous nociceptive pain states (46% vs. 98%, p=0.001). Conclusions. Pain is a relevant problem in patients affected by haematological malignancies in the advanced phase and its management can be effective and feasible by an experienced home care team, notwithstanding the high incidence of poor prognostic features, such as incident and neuropathic pain in this patient’s population.
PO-387
SINGLE PEG-FILGRASTIM INJECTION AFTER FLUDARABINE AND CYTARABINE COMBINATION FOR TREATMENT OF MDS AND AML: PRELIMINARY DATA ON HAEMATOLOGIC RECOVERY AND INFECTIOUS COMPLICATIONS
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Introduction. Regimens comprising fludarabine and cytarabine (FLA), with or without idarubicin, have shown promising results in the treatment of poor prognosis MDS and AML, with a favourable toxicity profile. In FLA regimens conventional G-CSF (G) is administered from day 0 to day 7 to induce cell cycling and sensitization to chemotherapy, then from day 12 to enhance recovery of neutrophils. PEG-filgrastim (PEG) is a covalently bound conjugate of filgrastim and monomethoxypolyethylene glycol. It has a longer elimination half-life than the unconjugated filgrastim because of decreased serum clearance. Notably, patients (pts) prefer injection of one single dose of PEG instead of daily G, when equivalence in hematopoietic recovery is demonstrated. We recently substituted G (300 mcg/sqm/day) with PEG (6/mg) at day 12 after FLA regimen in MDS and AML pts; here we report preliminary data on hematopoietic recovery after FLA with PEG compared to our historical data with daily G. Methods. we compared data from two groups of pts treated between 01/1999 and 03/2007.

Table 1. Characteristics.

<table>
<thead>
<tr>
<th>Group of treatment</th>
<th>PEG</th>
<th>G-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>N° pts</td>
<td>29</td>
<td>58</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>66 (21-74)</td>
<td>56 (18-72)</td>
</tr>
<tr>
<td>Diagnostic (WKO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML/MS</td>
<td>8 (27.6%)</td>
<td>13 (42.4%)</td>
</tr>
<tr>
<td>AMDS/AML</td>
<td>16 (55.2%)</td>
<td>27 (86.6%)</td>
</tr>
<tr>
<td>MDS/MDMS</td>
<td>5 (17.2%)</td>
<td>18 (51.0%)</td>
</tr>
<tr>
<td>N° of chemo cycles</td>
<td>33</td>
<td>71</td>
</tr>
<tr>
<td>Status before chemo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>untreated</td>
<td>17 (51.5%)</td>
<td>41 (57.7%)</td>
</tr>
<tr>
<td>DI/PR</td>
<td>7 (21.2%)</td>
<td>14 (19.7%)</td>
</tr>
<tr>
<td>active disease</td>
<td>9 (27.3%)</td>
<td>16 (22.6%)</td>
</tr>
<tr>
<td>Chemo cycles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLA</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>FLA + Ida</td>
<td>26</td>
<td>61</td>
</tr>
</tbody>
</table>

Table 2. Results.

<table>
<thead>
<tr>
<th>Group of treatment</th>
<th>PEG</th>
<th>G-CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filgrastim administered</td>
<td>median n° of vials (range)</td>
<td>1 (1-2)</td>
</tr>
<tr>
<td>Hematological CTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANC &lt; 500/mmc</td>
<td>n° days, median (range)*</td>
<td>16 (7-38)</td>
</tr>
<tr>
<td>Pts &lt; 20000/mmc</td>
<td>n° days, median (range)*</td>
<td>16 (5-70)</td>
</tr>
<tr>
<td>Duration of fever</td>
<td>n° of days/cycle, median (range)*</td>
<td>7 (0-18)</td>
</tr>
<tr>
<td>Infections/100 days</td>
<td>n° of cycles **</td>
<td>25 (7%)</td>
</tr>
</tbody>
</table>

* Mann Whitney U-test; ** Chi-square: p<0.05

Group PEG, 29 pts who received 33 FLA cycles, in which PEG s.c. injection was administered at day 12 (31 cases) or day 0 (2 cases); group G-CSF, 58 pts who received 71 FLA cycles, in which unconjugated filgrastim (dosage: 300 mcg/sqm/day) was given from day 12 until neutrophils recovery (>500/mmc). Hematological recovery was evaluated only in pts with a documented response to treatment (complete remission, CR, or partial remission, PR). Pts characteristics are summarized in Table 1. Median values of CTC grade 4 neutropenia and thrombocytopenia duration in the two groups were compared with the Mann Whitney U-test; cases of documented infections and episodes of fever of unknown origin (FUO) were reported and incidence in the two groups was compared with the chi-square test. Results. full results are shown in Table 2; in one case a second PEG-filgrastim injection was administered at day 32 for delayed recovery. Discussion. our preliminary results suggest that a single PEG-filgrastim injection after FLA regimens is equivalent to daily unconjugated filgrastim; haematological recovery and incidence of infective/FUO episodes during G4 neutropenia resulted comparable in the two groups of pts. Confirmation in a larger population of patients is needed. Moreover, as the single dose is preferred by pts to daily injections, the overall cost-effectiveness of the PEG-filgrastim formulation could prove favourable.

PO-388
HOME BASED CLINICAL ASSISTANCE AND QOL IN ONCOHAEMATOLOGICAL PATIENTS: ONE YEAR RESULTS OF HAEMATOLOGY UNIT, ASL NA 1 AND A.I.I. ASSOCIATION FLAVIANO MAGRASSI COOPERATIVE STUDY
U.O.C. Ematologia P.O. San Gennaro; 'Direzione Sanitaria ASL NA', Naples, Italy

Chronic oncohaematological patients have to be admitted frequently in hospital because of chemotherapy or supportive therapy. This condition leads, especially in elderly patients, to a negative impact on their quality of life (QoL). The Haematology Unit of San Gennaro Hospital, A.I.I. association, and ASI NA1 have activated an experimental public-private cooperation protocol starting 01/01/2005 to guarantee a haematological home based assistance after discharge in order to reduce hospitalization days and to improve the patients' QoL. After discharge 10 patients are enrolled to be treated at home by a team of haematologists provided by A.I.I. and nurses from the Haematology Unit coordinated by the head physician of the Haematology Unit. The patients can receive haematological checks, supportive therapy, non-aggressive cyclic chemotherapy. Inclusion criteria: previous admission in the Haematology Unit, haematological diagnosis, no acute complications at the time of enrolment, presence of a relative interacting with the patient and the assistance team, patient's residence in ASL Napoli 1 area, patient's written informed consent. The evaluation of the QoL is carried out through an EORTC QLQ-C30 questionnaire at the time of discharge, and then every three months. Since 01/01/2005, 10 patients (4 M and 6 F, average age 78 years range 66-88) affected by: Multiple Myeloma, Chronic Lymphocytic Leukemia, Myelodysplastic Syndrome, Essential Thrombocythemia have been enrolled. All patients received haematological home based assistance, checks and blood samples. 6/10 pts received cyclic chemotherapy, 4/10 pts received blood transfusion. At discharge the EORTCQLQ-30 questionnaire showed that the most distressing problems were insomnia, depression, stomach ache, apathy and anxiety. The health global evaluation scale of EORTC-QLCI-C30 at the end of the period (one year) showed an increase between 10-40 points in the patients' perception of own health. Haematological home based assistance is a modern approach with a favourable impact in patient's QoL. These results induced the Institutions to renew the protocol for another year.

PO-389
HOW TO IMPROVE THE QUALITY OF LIFE IN ONCOHAEMATOLOGICAL PATIENT WITHOUT ADDITIONAL COSTS
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The ASL NA1 is organized in 10 districts, corresponding to the several UU.SS.LL of the City-Hall of Naples. The San Gennaro Hospital is one of the nine Hospitals in the ASL NA1. The operative unit of Haematology of the San Gennaro Hospital is able to hospitalize 13 patients and
other 4 patients in Day Hospital regimen. In 2004 798 patients have been accepted for a total period of 6890 days of hospitalization. The total gain was 5,400,000 euros. The project of assistential continuity will be developed between 2005 and 2007. Starting 01/01/2005 ten patients who had total 1000 hospitalization days have been enrolled. They have been assisted by 2 haematologist MDs and 2 nurses in home-based regimen for a total of 200 monthly hours. We plan to obtain 30% decrease in the hospitalization days compared to previous year for enrolled patients, 7% increase in the turnover of the patients and consequent decrease in the waiting list. The financial objective was planned to be an increase of 7% of the gains obtained by DRG per year, thus compensating the costs of home-based clinical assistance. One year activity Results. We observed 44% decrease in hospitalization days of enrolled patients (527 days against 1000) and 14% increase in new admitted ones. The gains obtained by DRGs per year have gone up in 14%. Since the reduction of the hospitalization days for a patient induces an amelioration both of his quality of life and the reduction of the risk of nosocomial infections, the improvement of the quality of life of the patient could be obtained without extra charges.

PO-390
CONTROLLED-RELEASE (CR) OXYCODONE FOR THE TREATMENT OF BORTezOMIB-INDUCED NEUROPATHIC PAIN IN PATIENTS WITH MULTIPLE MYELOMA  
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Background. Iatrogenic neuropathic pain is a distressing symptom frequently affecting the quality of life of patients affected by hematological malignancies. The proteasome inhibitor Bortezomib, a very effective agent for the treatment of multiple myeloma, has been reported to induce a painful axonal sensitive neuropathy. Aims: an observational trial to evaluate the effectiveness and the safety of CR oxycodone for the treatment of Bortezomib-related neuropathic pain. Patients and Methods. Seventeen patients affected by myeloma (median age 62 yrs.) with bortezomib-related pain received oral CR-oxycodone at the starting dose of 10 mg bid. Eight pts. were assuming NSAID analgesics, 7 anticonvulsivants 7 weak opioids. Pain intensity was evaluated by using the numerical rating scale (NRS) along with the global patient evaluation of efficacy (GPE). CR Oxycodone was started at 10 mg bid and titrated according to the presence of numerous painful syndromes, not answering to both his quality of life and the reduction of the risk of nosocomial infections, the improvement of the quality of life of the patient could be obtained without extra charges.

PO-391
TREATMENT OF PAINFUL SYNDROMES WITH CONTROLLED-RELEASE OXYCODONE IN HEMATOLOGIC PATIENTS  
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Background and Aim. Many malignant hematologic malignancies record the presence of numerous painful syndromes, not answering to the employment of anti-inﬂammatory (NSAIDs) or antiepileptics drugs. Aim of this study has been to estimate the effectiveness of opioid drug (CR-oxycodone) in the treatment of pain from different causes. Patients and Methods. 31 pts. (47%) were affected by multiple myeloma 14 (21%) lymphoma, 17 (26%) leukaemia and 3 (5%) other syndromes; pain was caused by localization of malignant disease in 29 patients (44,6%), iatrogenic toxicity in 24 patients (37%), Herpes Zoster neuropathy in 4 patients (6%), opportunistic infections in 7 patients (11%) and several causes in one patient (1,4%). Pain intensity was evaluated by using the numerical rating scale (NRS) along with the global patient evaluation of efficacy (GPE). CR Oxycodone was started at 10 mg bid and titrated with oral morphine. The 97% of patients had previous treatment judged not effective (22,9% strong opioids, 26,5% week opioids, 24,5% NSAIDs, 21,1% anticonvulsivants, 5,2% others). Results. The treatment period was 14 days; average NRS 7,56 at basal time, 3,81 on day 3, 1,9 on day 7 and 0,98 on day 14. All the patients have been treated with an average dosage of 32,91 mg/die oxycodone CR (range 20-80 mg/die). GPE was evaluated good/excellent in 79,2% of patients, satisfactory in 7,5% and unsatisfactory in 3,5% (10% of patients didn’t respond). Rescue doses were administered in 80% of patients during first week of treatment while only 25% used rescue dose during the third week. In particular, 18,6% of used drugs was NSAIDs 18,7% weak opioids and 62,7% strong opioids. The side effects remarked were of poor intensity; only a patient has suspended the treatment. Discussion: Oxycodone CR has conﬁrmed of being an effective drug in the treatment of pain correlated to hematological tumours.
PO-393
TOTAL PARENTERAL NUTRITION FOR PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION: COMPARISON OF STANDARD, GLUTAMINE ALONE, FISH-OIL N-3 FATTY ACID ALONE AND GLUTAMINE PLUS FISH-OIL N-3 FATTY ACID ENRICHED REGIMENS

Background. Glutamine (GLN) is one of the most important substrate for the glutathione’s synthesis and for the growth of fibroblasts, lymphocytes and enterocytes. On the other hand, the fish-oil-derived n-3 fatty acids (n-3FA) have been referred to interfere with inflammatory and immune response by regulating cytokines synthesis. Materials and Methods. From 2000 to 2002, 29 patients undergoing autologous SCT for malignant diseases entering the study comparing standard total parenteral nutrition (TPN) regimen (Group A: n = 16) with a TPN regimen containing GLN at dose of 0.22g/kg/day (Group B: n = 13). The patients in the 2 groups were comparable for age, diagnosis, pre-transplant therapy and conditioning regimens. Isonitrogenous and isocaloric regimens containing GLN at dose of 0.22g/kg/day plus n-3FA at dosage of 1.5 mL/kg (Group C: n=19) or TPN containing n-3FA alone at the same dosage (Group D : n = 18). Patients of group C and D were retrospectively compared with the other two groups (A and B) for a number of variables: length of hospitalization, number days to PMN engraftment, duration of febrile neutropenia, number days on TPN, grade of mucositis. Antimicrobial or antifungal therapy, days to PMN engraftment and grade of mucositis.

Results. As compared to groups A and B, groups C and D were significantly associated with a shorter hospitalization (p<0.01). No difference was observed between group C and D, but patients of Group C were on TPN for a number of days significantly lower (p<0.01). Group C and D experienced lower incidence and duration of febrile episodes during neutropenia and, consequently, a reduced number of days on antibiotic or antifungal therapy, but these differences were not statistically significant. Moreover, a lower incidence and severity of mucositis was observed in group C and D as compared to group A and B, with no difference between group C and D. No difference was observed for number of days to PMN engraftment. Conclusions. Our retrospective study suggests that in patients undergoing an autologous transplant fish-oil n-3 fatty acid, alone or associated with GLN, could be a relevant component of TPN in order to improve the general outcome of the patients.

PO-394
QUALITY OF LIFE, ANXIETY AND DEPRESSION IN AUTOLOGOUS OR ALLOGENIC STEM CELL TRANSPLANT PATIENTS HOSPITALIZED IN ISOLATION REGIMEN
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Introduction. Aim of this study is the evaluation of the emotional/psychological status and of the quality of life of a sample of patients hospitalised in isolation rooms in the Bone Marrow Transplant Unit of the Verona University hospital for autologous or allogenic stem cell transplant. Our main task is to explore whether anxiety and depression arising during the hospitalisation are related to the personality traits and to emotional disorders and whether the changes of mood are linked to the medical treatment. Furthermore, the quality of life of these patients is assessed in order to verify if this variable tends to modify according to their health status, the mood and treatments delivered. Methods. This is a prospective longitudinal survey with a one-year recruitment period. Patients who enter the study, previous written informed consent, will be assessed before and at the admission to the hospital, during the hospitalization (once a week) and six months after the discharge. At the baseline the patients fill a set of standardised measures, which include: Cognitive Behavioural Assessment (CBA) in which state and trait anxiety, personality, stress, fears, depression and obsessive and compulsive traits are explored. During the weekly assessments the patients fill the State-Trait Anxiety Inventory (STAI-X1, STAI-X2), the General Health Questionnaire (PHQ), evaluating the subjective perception of health, the Zung Depression Rating Scale (SDS), exploring the depression prevalence. Six months following the last inpatient evaluation, the same instruments are administered along with Ferrans and Powers Quality of Life Inventory, exploring the general quality of life and the quality of life according to the health and psychological status, to the social-economical condition and family involvement. The physician fills a schedule reporting the information given to the patients regarding his medical status and a form describing the treatments delivered. Statistical analysis. The changing in scoring is evaluated by the t test, Wilcoxon and Friedman; the predictors are estimated linear hierarchical models. Discussion. The recruitment started on July 2006. At present 57 patients have been recruited and evaluated before and during the hospitalization. Of these, 18 subjects have been evaluated also six months after the discharge. The recruitment will be closed on June 2007. During the Congress we will present our data about the association between the quality of life and the anxiety disorders and depression of these patients.

PO-395
ZOSTER-RELATED PAIN IN HAEMATOLOGICAL MALIGNANCIES: PROMPT AND STABLE RELIEF OF REFRACTORY PAIN SYNDROMES BY OXYCODONE
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Background. Herpes Zoster Virus (HZV) outbreak and post herpetic neuralgia (PHN) portray significant morbidity in patients affected by lymphoproliferative disorders (LPD) and in those submitted to haematopoietic stem cell transplantation (HSCT). Early treatment of acute zoster pain (AZP) can reduce the incidence of PHN. Pain refractory to standard agents may represent a difficult concern. We treated with Oxycodeone 10 consecutive HZV-pain patients unresponsive to several analgesics agents (Table 1).

Table 1. Zoster-related clinical features and pain outcome

AZP: acute zoster pain; PHN: post herpetic neuralgia; F: female; M: male; NWL: non Hodgkin lymphoma; ALL: acute lymphoblastic leukaemia; AML: Acute Myeloblastic Leukaemia; MRD multiple myeloma; PGB: pregabalin; GB: gabapentin; NSAID: Non-Stomadial Anti-Inflammatory Drugs; PMN: paracetamol; AT: amitriptyline. *Reduction of almost 50% of pain rate with respect to the baseline level. OXY: oxycodone.

Case series. First PHN patient was a woman with a PHN diagnosed 30 months before. About three years later she has suffered from painful shingles and has received acyclovir and gabapentin. Given the persistence of neuropathic complaints, after three months gabapentin was...
replaced by high doses of pregabalin without any benefit. Given the lack of response to pregabalin alone, this agent was reintroduced by us at standard dose (150 mg/day) in addition to tramadol (200 mg twice daily). Only transiently pain relief was achieved for which tramadol was replaced with oxycodone that was titrated until 10 mg thrice daily, allowing a stable control of pain. The second PHN patient was a man affected by acute lymphoblastic leukaemia who received oxycodone because of a severe PHN lasting from 4 months, achieving a rapid and stable pain relief. Patients 3 was affected by acute myeloblastic leukaemia and presented PHN afflicting the trigeminal region. He was unresponsive to pregabalin and tramadol, which was replaced by oxycodone in escalating dose until an acceptable pain relief. Patients 4 to 10 were affected by LPD, for which they have received several cytotoxic regimens, including long term steroids. They presented similar herpetic clinical features, receiving antivirals associated with non-opioid analgesics and with gabapentin or amitriptyline without significant benefits. We successfully treated them with combination of gabapentin-oxycodone without any side effect and, remarkably, none of them developed PHN after a median follow-up of 10 (3-20) months. Conclusions. Convincing evidences of provided benefits of opioids in this setting have been demonstrated. In particular, oxycodone and tramadol were reported as effective to relieve neuropatic pain. Our experience suggests that: 1) an opioid should be offered for painful HZ outbreak or PHN even when tramadol failed in relieving pain; 2) a prompt intervention is highly recommendable in the aim to prevent PHN and, in this view, oxycodone can represent a suitable option.

PO-396
DEPLETIVE LEUKAPHERESIS IN PATIENTS WITH ACUTE HYPERLEUKOCYTIC LEUKAEMIA
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Introduction. Hyperleukocytosis in leukaemia patients is defined as white blood count (WBC) greater than 100,000 cell/microliter. It is frequently associated with poor prognosis and early mortality. These patients often present leukostasis and endothelial damage due to sludging of leukaemic blasts in capillary bed and to reciprocal adhesive interactions. Depletive leukapheresis has a role as initial treatment to allow induction chemotherapy. We retrospectively analyzed the effectiveness of depletive leukapheresis at our institution. Methods. From 1998 to 2006 we admitted twenty-three patients with hyperleukocytic acute leukaemia. The patients were 11 males and 12 females with a median age of 55 years (8-90). All patient underwent leukapheresis within 12 hours from admission using continuous-flow separators. We performed daily procedure until peripheral WBC count was <100,000 cell/microliter or patient’s clinical status improved to allow the start of chemotherapy. Results. We treated 23 patients with acute leukaemia: 4 had lymphoblastic leukaemia, 2 had M1, 7 had M2, 3 had M4, 2 had M5b, 2 had secondary leukaemia and 2 had no subtype classification according to FAB classification. Initial median WBC count was 228,000/microliter (129,000-427,000). Eight patients showed respiratory distress, four neurological symptoms and seven renal failure. Thirty-two leukapheresis procedures were performed with Cobe Spectra (Gambro BCT) or Fresenius AS204 (Fresenius) using peripheral venous access. Fifteen patients underwent one procedure, seven underwent two procedures and only one patient required three procedures. Processed total bone marrow volume was 6,800 mL (3,800-10,900) in a median time of 175 minutes. In two patients the procedure was stopped because of psychological status. The median percentage of WBC reduction after the first leukapheresis was 41% (19-67). Discussion. In our experience leukapheresis procedures appear to be a safe and efficient method for fast leucocyte reduction and are well tolerated also in critical setting. One single procedure has been sufficient to improve clinical condition in 65% of patients. A second or third procedure was necessary when at the admission WBC count was higher than 250,000/microliter. We confirm that leukapheresis has a role in treating hyperleukocytic patients. Further data are needed to definitively prove its efficacy on early mortality rate reduction and overall survival.

PO-397
EFFICACY AND COST ANALYSIS OF PALONOSETRON (PLS) AND TROPISETRON (TPS) USE IN CHEMOINDUCED NAUSEA AND VOMITING (CINV) IN HEMOPHATIC PATIENTS
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Introduction. CINV incidence is 60%-90% with charbonplatin and high dose cytarabine, 30-60% with ifosfamide and idarubicin and 10-30% with cytarabine (inferior/equal to100mg) and daunorubicin. 5-HT3 antagonists as TPS and PLS have a prolonged efficacy (half-life of 8 and 40 hours respectively). Aim of the study is to evaluate clinical efficacy and pharmacoeconomic impact of PLS and TPS in patients receiving 5-7 days chemotherapy. Methods. We evaluated 57 patients treated with 5-7 days chemotherapy for NHL(22) or AML(15). M/F was 16/21, median age was 68 years (R25-81). NHL patients, receiving ifosfamide or high dose cytarabine and charbonplatin, were at high-risk of CINV. For each patient antibiotic and opioid administration, anticipatory emesis, early or late emesis, mucositis, gastropathy, esophagitis and diarrhoea presence, CINV-related therapy and meal stop were evaluated. A cost analysis was performed considering the median of global antiemetic expense for each patient. Subsequently only the median of CINV-related antiemetic expense was considered. Patients receiving the same chemotherapeutic regimen were randomized in two groups: one receiving PLS and the other TPS. This is a monocentric, prospective, randomized study. PLS was given only at first chemotherapy day, while TPS was given from one to three times/day. Results. In TPS group M/F was 8/9, median age 68 years (R25-81). AML/NHL was 8/12. Seven patients received antibiotic therapy during antiemetic administration. Four patients showed CINV. In PLS group M/F was 8/10, median age 68.5 years (R31-79), AML/NHL was 8/10. Ten patients received antibiotic therapy during antiemetic administration. Three patients showed CINV (two of them with gastritis and anticipatory nausea responding to methiclopramide administration). Fisher exact test (p=0.69), Odds Ratio (1.5, CI95%:0.3-8) and relative risk (0.7, CI95%:0.18-1.7) didn’t showed significant differences in CINV incidence between PLS and TPS group. In PLS group, median global antiemetic expense for each patient was 107.25euro (R107.25-384.53), while in TPS group was 393euro (R30-515). Median antiemetic expense only for real CINV, was 107.25euro (R107.25-290) in PLS group, while in TPS group was 393euro (R30-515). Discussion. PLS efficacy is not inferior to TPS in CINV prevention in patients receiving multiple day chemotherapy regimens. In pharmacoeconomic analysis the median cost of PLS treatment is about 300euro inferior to TPS treatment cost.

PO-398
REDUCED-DOSE RASBURICASE IN THE TREATMENT OF HYPERURICEMIA IN LEUKEMIAS AND LYMPHOMAS PATIENTS
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Tumor lysis syndrome (TLS) is life threatening metabolic complication of haematological malignancies with a large tumour burden and rapid cell turn-over. Hyperuricemia, hyperphosfatemia, hyperkaliemia and hypocalcemia are the main biochemical abnormalities of TLS that may lead to serious complications such as renal failure, cardiac dysrhythmias, and death. Hydration, urinary alkalization, and Allopurinol are the standard of care in the treatment and prevention of hyperuricemia. Allopurinol action is rather slow in reducing U.A. concentration, because acts on the new synthesis of U.A. and not on pre-existing one, hence, the decrease in U.A. is reached several days after the therapy. Rasburicase, urate-oxidase from recombinant DNA catalyzes the oxidation of already synthetized uric acid into allantoin. It rapidly reduces uric acid levels in 4 hours, it is a new alternative for the management of hyperuricemia in leukemias and lymphomas patients. Rasburicase recommended dose is 0.20 mg/Kg/die/ iv daily for 5-7 days. However, a large number of studies have suggested that different doses, lower than standard dose and for shorter period than recommended, are effective. We report data about 38 adults 26 M, 12 F, mean age 56 years (range: 17-84), with hematologic malignancies and hyperuricemia median U.A. levels 12.1 (range 8.5-17.4 mg/dl) who received a 6 mg dose of Rasburicase on day 1 and a 3 mg dose on day 2 started the day -1 of chemotherapy. All
phases, may confer them the connotation of oligarchic organization. This kind of structure may predispose to some inconveniences, regarding especially the people who were excluded from the trials management. In order to clarify any positive or critical situation in an hypothetic National Onco-Hematologic Cooperative Group, a survey was carried out, in particular to determine the nature and frequency of those criticals and to try to find solutions. The survey was conducted during a Master in Health Services Management in Haematology that took place at the ISTUD in Stresa between 2005 and 2006. A questionnnaire consisting especially of the people who were excluded from the trials management and non academic Onco-Hematology departments. The questionnare was submitted to 75 health operators involved in clinical trials, 63 of which were graduates (29 with and 12 without executive responsibilities, 21 trainees) and 12 were not graduates (nurses, data manager). At first, there was a substantial opinions uniformity on most queries, concerning all categories. Thus the questionnare seems to provide significant information for most of the interviewed people who deal with clinical research. Briefly, the participation to a cooperative group gives the opportunity to compare personal experiences with other colleagues and to share informations that could significantly improve health cares quality and operators knowledge. Moreover, the involvement in clinical trials determines a cultural growth and a greater scientific regard, estimation and prestige of the individuals and of the institutions. Scientific prestige desire is however not completely satisfied. Minor centres and physicians without managing responsibilities need particularly groups and cooperation and esteem. A further involvement in planning and proposing the trials is one of the most important causes of this dissatisfaction. To improve the scientific prestige means also to increase the collective character of the authors, who must be globally cited in scientific publications that should be more numerous. Another way to satisfy these ambitions could be the conception of sub-studies that can involve more the participants. Negative elements seem particularly the absence of a real dialogue between all the participating Centres and, in addiction, oligarchic decisions, clash of power and, sometimes, scarce organization. Generally, costs dont seem a limit, although sometimes major financing could be crucial. The work increase determined by clinical trials is noteworthy: the majority of the interviewees declare a 40% increase. A significant percentage of the interviewees considers important to create professional figures (such as data manager, trial office) with a specific role in managing clinical trials. It is also necessary to qualify activities organization drawing particular attention to team work, identifying the specific role of a single cooperative group and of the single member of the group. An increased number of periodic meetings and an improvement in the use of internet to divulge and promote clinical trials are retained useful to ameliorate the involvement. Unexpectedly, it was not considered noteworthy the opportunity, offered by the clinical trials, to obtain drugs that would be available on the market only 2-4 years after.

**PO-401**
**FEBRILE NEUTROPIA IN ELDERLY PATIENT-ACUTE MYELOID LEUKAEMIA: MONOCENTRIC PHARMACOECONOMIC ANALYSIS**

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**Background.** More than 40% of patients with acute myelogenous leukaemia (AML) are over 65 years old at the time of the diagnosis. Which is the best treatment for AML in elderly patients is still debated. Actually there are three main options: supportive treatment (ST), standard dose chemotherapy (SDC) and low dose chemotherapy (LDC), with different risk of febrile neutropenia. Among patients over 65, the mortality rate during the aplastic phase that follows intensive chemotherapy is 30 to 40%. Infections diagnosed or of unknown origin during febrile neutropenia (FN) cause approximately two thirds of treatment-associated deaths. FN involves from 50 to 90% of elderly patients. **Aims.** Aim of this study is to analyze the pharmacoeconomic impact of ST, SDC and LDC on antimicrobial treatment and outcome of FN in elderly AML patients. This is a retrospective, nonrandomized study. The analysis was performed on 16 multiple choice queries was distributed in 4 Italian academic and non academic Onco-Hematology departments. The questionnaire was submitted to 75 health operators involved in clinical trials, 63 of which were graduates (29 with and 12 without executive responsibilities, 21 trainees) and 12 were not graduates (nurses, data manager). At first, there was a substantial opinions uniformity on most queries, concerning all categories. Thus the questionnare seems to provide significant information for most of the interviewed people who deal with clinical research. Briefly, the participation to a cooperative group gives the opportunity to compare personal experiences with other colleagues and to share informations that could significantly improve health cares quality and operators knowledge. Moreover, the involvement in clinical trials determines a cultural growth and a greater scientific regard, estimation and prestige of the individuals and of the institutions. Scientific prestige desire is however not completely satisfied. Minor centres and physicians without managing responsibilities need particularly groups and cooperation and esteem. A further involvement in planning and proposing the trials is one of the most important causes of this dissatisfaction. To improve the scientific prestige means also to increase the collective character of the authors, who must be globally cited in scientific publications that should be more numerous. Another way to satisfy these ambitions could be the conception of sub-studies that can involve more the participants. Negative elements seem particularly the absence of a real dialogue between all the participating Centres and, in addiction, oligarchic decisions, clash of power and, sometimes, scarce organization. Generally, costs dont seem a limit, although sometimes major financing could be crucial. The work increase determined by clinical trials is noteworthy: the majority of the interviewees declare a 40% increase. A significant percentage of the interviewees considers important to create professional figures (such as data manager, trial office) with a specific role in managing clinical trials. It is also necessary to qualify activities organization drawing particular attention to team work, identifying the specific role of a single cooperative group and of the single member of the group. An increased number of periodic meetings and an improvement in the use of internet to divulge and promote clinical trials are retained useful to ameliorate the involvement. Unexpectedly, it was not considered noteworthy the opportunity, offered by the clinical trials, to obtain drugs that would be available on the market only 2-4 years after.
SDC, 6 with LDC, 7 with ST. 14 patients presented comorbidity, 13 had PS 0-1, 8 had secondary leukaemia and M2-M4 were the most represented FAB subtypes. The most frequent comorbidities were diabetes (7 pts), second neoplasms (5 pts) and ischemic cardiopathy (4 pts). Results. Global median survival for all patients, without regard for the treatment received, was 8 months (R1-10). Median survival was 5 months for patients treated with SDC, 5.5 for LDC and 1 for ST. Median hospitalisation was 1 month for ST (R0.5-1), 2 months for SDC (R1-5) and 1 month for LDC (R0.2-5). The antibiotic expense was higher in ST (euro9555/month vs euro1167.40/month in SDC and euro279.55/month in LDC) and G-CSF administration wasn’t effective in antibiotic expense reduction (euro1800/month vs euro1100/month in patients without G-CSF). Death for sepsis were: 5(100%) in SDC, 1.6(16%) in LDC and 3.7(43%) in ST group. Empirical antifungal therapy was used in 5(100%) SDC, 2/6(33%) LDC and 3/7(43%) ST patients. Summary/conclusions. This work shows that treatment type used in elderly AML interacts not only with disease, but also with patients’ body (increased mucositis, organ toxicity) and with immune system (more or less pronounced iatrogenic immunosuppression), conditioning the economic expense and the outcome of FN and AML. Therefore AML treatment type is to be considered an essential component of FN therapy. LDC seems to be an economic and effective therapeutic option for elderly AML and FN connected to it, especially if performed in outpatient setting. Nevertheless these data need further confirmation on a larger patient cohort.

**PO-402**

**A SINGLE-CENTER ANALYSIS OF TREATMENT OUTCOMES AND SURVIVAL IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES 1994-2006**


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**Background.** Survival of neoplastic patients is generally calculated on the basis of therapeutic protocols which include selected patients, or on the basis of Register data which include data coming from a more or less wide country. (1,2). **Material and Methods.** In order to verify the real life-expectancy of patients with hematologic malignancies diagnosed, treated and followed in our Unit, we performed a retrospective analysis of all the patients with hematologic malignancies come to our observation since 1994. All the patients were included, irrespectively of comorbidity or other factors. **Results.** 90 Hodgkin Disease (HD) pts had a median follow-up (F.U.) of 54 months (1-156). Their median survival was not reached after 150 months of maximum observation. Older (>60years) patients had a median survival of 51 months, while younger ones did not reach the median line. 384 Grade Non Hodgkin Lymphoma (L NHL) pts had altogether a median survival of 129 months (99 months for older and not reached for younger pts), with a median F.U. of 49 months (1-156 months). (Figure 1-A). 407 High Grade NHL (HG NHL) had a median survival of 112 months (53 for older and not reached at 156 months for younger ones), with a median F.U. of 32 months (1-156 months). (Figure 1-B). 249 B-cell Chronic Lymphocytic Leukemia (B-CLL) pts had a median F.U. of 45 months (1-156 months). Median overall survival was 99 months (76 months for older pts and not reached for younger ones). 174 Acute myeloid leukaemia pts, with a median survival of 11 months (9 months for older and 25 months for younger pts) with a median F.U. of 10 months (1-132 months). (Figure 1-C). 24 Acute lymphoblastic leukaemia pts with a median follow up of 16 months (1-96 months). Overall median survival was 18 months (5 for older and 19 for younger pts). The 318 Multiple Myeloma pts, with a median follow up of 32 months (1-156 months), showed a median survival of 64 months (50 for older and 89 for younger pts). All the patients with a plasma-cell infiltration of the bone marrow >20% were included. Part of them have never been treated. **Conclusions.** These data have been compared with those included in the Istituto Oncologico Romagnolo Registry. We found a difficulty in comparing the data due to the old nomenclature in the Registry. Therefore, this study will be useful in standardizing the criteria of inclusion of the patients. **Acknowledgements.** This study was carried out with the support of the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (I.R.S.T.), Meldola-Forlì and of the Ravenna section of the Associazione Italiana contro le Leucemie, Linfomi e Mieloma (Ravenna A.I.L.).

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Figure. Survival curves of patients with different hematologic malignancies.
QUALITY OF LIFE IN AML PATIENTS OVER 70 YEARS

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The treatment of acute myeloid leukaemia in elderly with age > 70 years is still debated. In literature numerous studies have valued the feasibility of intensive chemotherapy in these patients, but few studies have really valued the quality of the life of this group of patients. The aim of the study and to value the quality of the life of these patients considering the days of hospitalization, the EFS and the OS of 2 groups of AML elderly patients treated with intensive chemotherapy (IC) or with low doses aracyn and/or support (M). From June 2001 to May 2007 we have treated in our Division 48 AML patients, 24 male and 24 female with median age of 78 years (70-90 years). 15 patients (7 M and 8 F with median age of 73 years) have received intensive chemotherapy (I.C. Flag and MICE) and 33 (17 M and 16 F with median age of 79 years) have received maintenance (24 patients low dose cytarabine and/or 9 patients support). In IC group 5 patients (33%) have obtained to complete remission (CR) with to EFS and OS media of 4, 78 and 6,3 months respectively, the rate of TRM has been of 27%. In the M group the CR has been documented in 9 patients (37%) with to EFS and OS media of 5,16 and 5,9 months respectively (Figures 1-2). The media days of hospitalization has been of 34 and 11 respectively for IC and M group, such difference is statistically significant (p: 0.000001). These results have not shown difference in terms of CR, EFS and OS among the two groups of patients while has been statistically shown a significant difference in the days of hospitalization. In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients, but it perhaps engraves on their quality of the life. A new therapeutics strategy is necessary for to improve the EFS and OS and quality of life in these patients. Interesting it is the use of new drugs, to improve the results in this group of patients, and to give less suffering, engraving on their quality of the life, reducing the necessity of hospitalization.

EVALUATION OF QUALITY OF LIFE IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

SQUID Study Group (Survey on Quality of life in myeloDysplasia)
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Introduction. Patients with MDS are at continuous risk of complications and must face the reality of a relatively low survival while depending mainly on supportive care. The disease’s chronic nature, the probability of progressive evolution and the rapid changes in physical status makes the MDS patient unique within the cancer population. Anemia and fatigue are frequently associated with MDS and a variety of associated symptoms may influence the patients’ daily life. Quality of life (QoL) in MDS is undoubtedly compromised by physical, functional, psychological, social, and disease-specific components. We have designed a prospective survey to evaluate the correlation between QoL, hemoglobin levels and other disease-associated variables in patients affected by MDS from diagnosis. The comparison between the patients’ and the physicians’ perception of QoL and the QoL changes related to adaptation, progression and treatment response shall be evaluated throughout the investigation. Baseline characteristics of the MDS population at diagnosis will also be provided. Methods. This is an 18-month prospective observational investigation. One hundred and fifty consecutive MDS patients at diagnosis with IPSS score < 2 and at least one cytopenia will be included in the evaluation. Demographic and disease-specific data will be collected and QoL will be evaluated by the QoL-E questionnaire completed by patients and respective physicians. Study visits will be performed monthly until week 12; the subsequent visits will be performed at 6, 12 and 18 months. The association between QoL and Hb values will be analyzed in an univariate manner and by multiple regression analysis based on all the observations and including the factor time in the model. Results. The enrolment started in February 2007 and is planned to end in December 2007. The survey is currently ongoing in 14 Italian centres (SQUID Study Group) and results may furnish novel insights in health- and treatment related QoL in MDS.
Treatment with Depocyte®, in accordance with what is already reported in literature is an effective treatment for therapy and prophylaxis of CNS localizations in haematological malignancies. Its gradual release formulation allows a lower frequency of IT administrations than traditional drugs; moreover, it has shown a good handleness and absence of adverse effects in all the patients treated.

**PU-002**

**CAUDA EQUINA COMPRESSION BY ISOLATED RELAPSE OF LYMPHOPLASMACYTIC LYMPHOMA**

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A 42 years-old woman was admitted to our day-hospital with a 4-month history of vulvodynia and successively pain in the lower back resistant to common analgesic drugs. Previous medical history included: 1) diagnosis of Lymphoplasmacytic Lymphoma with IgM paraprotein 7 years later treated with CNOP chemotherapy and autologous peripheral blood stem cells transplantation; 2) recurrent lumbal pain due to dumbbell lumbal disc hernia (L3-L4; L5-S1). Neither palpable lymphadenoephyphism nor organomegaly was present on physical examination. Abnormal neurological findings included sensory impairment of the L5-S1 distribution. Lumbo-sacral column and pelvis radiographs were normal. A MNR of the lumbo-sacral spine and pelvis disclosed a mass into the spinal canal extended from S3 to S5. Bone marrow examination was normal. The initial therapeutic approach consisted in surgical debulking of tumour; the histopathological diagnosis of Lymphoplasmacytic Lymphoma was made after examination of the specimens obtained from the operation. Radiotherapy began 3 weeks later; the patient was given 36 Gy in 18 fractions from a linear accelerator to the site of sacral tumour. Successively weekly Rituxumab 375 mg/m² was administered 4 times. Treatment plan was completed by 4 intrathecal administrations of Methotrexate 12 mg t.d. The patient is alive and well today, 18 months after the sacral relapse of Lymphoplasmacytic Lymphoma. Cauda equina compression by malignant lymphoma is infrequent, with an incidence inferior to 10% in the literature. Any cancer capable of haematogenous spread can produce spinal cord localization and the latter can occur also through direct tumour extension. Spinal cord and radicular involvements are frequent events in the natural history of NHL, which usually are secondary to invasion of spinal extradural space, and rarely are the initial manifestations of this disease. Primary epidural lymphoma accounts for less than 10% of epidural tumours and less than 1% of NHL. Thoracic segment is predominantly affected, but any spinal region can be involved. Diagnosis is based on histological analysis of tissue taken during the surgical operation. Histologically the spinal cord lymphomas are more frequently high-grade NHL. It is well known that cauda equina syndrome can be caused by vertebral lesions and primary spinal tumours; despite his rarity, it is still necessary to keep in mind malignant lymphoma in case of neoplastic involvement of the cauda equina.

**PU-003**

**IMMUNOCHEMOTHERAPY REGIMENS FOR THE TREATMENT OF YOUNG PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA**


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B-cell chronic lymphocytic leukemia (CLL) has traditionally been considered indolent, with a prolonged clinical course. However, a large proportion of patients with CLL often require more immediate treatment of their leukaemia. Many studies indicate that cytotoxic therapy based on alkylation agents or fludarabine, in the indolent phase of disease, does not prolong the survival time of CLL patients. The mononclonal antibodies directed against CD20 antigen (alemtuzumab, Campath-1H) and CD20 antigen (rituximab) demonstrate also considerable activity in CLL patients. The use of rituximab with the cytotoxic agents cyclophosphamide and fludarabine (FC-R) has achieved complete remission (CR) with no detectable CLL, as assessed by minimal residual disease (MRD) techniques, in a significant proportion of previously untreated and previously treated CLL patients. Monotherapy with alemtuzumab has also been shown to achieve a complete response with undetectable MRD in several patients with relapsed/refractory disease. We have investigated, in a small cohort of young untreated CLL patients, the feasibility and effectiveness of a combination therapy using alemtuzumab consolidation to improve the quality of response to FC-R induction. In our institution we treated 12 patients (4 F and 8 M; median age: 45 years, r.: 35-52 years; Rai stage III-IV) with 6 cycles of FC-R (fludarabine at a dose of 25 mg/m² i.v. on days 1-5, cyclophosphamide at a dose of 250 mg/m² i.v. on days 1-5, rituximab at a dose of 375 mg/m² on day 0)). One month after the last cycle all patients were subjected to a disease restaging that showed a clinical CR, but 9 out of 12 patients showed the presence of MRD in the bone marrow. Therafter all patients received, after an initial dose escalation over 3 days, alemtuzumab 10 mg subcutaneously three times per week for 12 weeks. Cytomegalovirus reactivation occurred in 10 patients, all of whom were successfully treated with oral valganciclovir. At a clinical re-staging performed after one, three, six and twelve months from the end of therapy 10 out of 12 patients showed a CR with undetectable MRD (molecular CR). FC-R is highly active as initial therapy also in young CLL patients. However, a consolidation therapy with alemtuzumab seems to be required for achieving a stable molecular CR. Moreover our preliminary results show acceptable toxicity profile of this therapeutic approach.
From December 2000 to November 2006, 30 pts (18 females and 12 males) median age 43.4 years (range 18-66) underwent NST because of high-risk Hodgkin Disease (9), non Hodgkin Lymphoma (18) and Chronic Lymphocytic Leukaemia (3). Disease status at transplant was as follow: 5 in CR (3 NHL, 2 HD) 21 in PR (13 NHL, 7 HD, 1 CLL) and 4 (3 NHL, 1 HD, 1 CLL) in PD. In 26 cases, grafts were mobilized from HLA identical sibling donors, while in 4 stem cells were obtained by a matched unrelated donor. Conditioning regimens consisted of Fludarabine, Thiopeta and Cyclophosphamide in 18 cases, Fludarabine and Cyclophosphamide in 5, Campath-1, Fludarabine, Melphalan and TBI in 2, Fludarabine, Cyclophosphamide and Rituximab in 1, Fludarabine and Thiopeta in 1, Fludarabine, Melphalan, Thiopeta and ATG in 1, Fludarabine, ATG and TBI in 1, TLI and ATG in 1. Cyclosporine–A (CyA) and Methotrexate (MTX) were used as Graft Versus Host Disease (GvHD) prophylaxis in all cases but two, where Campath-1 and Mo Rafael monoclonale (MM-F) combined and one is employed prophylaxis with CyA and MM-F. A mean number of 5.49×10^6/Kg CD34+ cells (range 3.4-7.7) were infused. Pts received a mean of 3.4 (range 0.22) packed red blood cells, while platelet support was apheresis (median 2,4, range 0-10) or platelets (median 2,4, range 0-10) or randomly obtained platelet concentrates (median 7.5, range 0-32). 4 pts experienced mucositis WHO grade 3-4, 5 patients WHO grade 2, 7 pts WHO grade 1, while the remaining 14 pts did not show sign of mucositis. 18 pts had fever (11 F/U and 7 microbiologically documented). All pts engrafted at median day +12,1 (range 6-19) for PMN, +22 (range 15-30) for platelets and +30 (range 20-40) for aGVHD. 3 pts developed a WHO grade 1 cutaneous aGVHD, a WHO grade 3 cutaneous aGvHD was seen in 1 pts, a WHO grade 4 liver aGVHD was seen in 2 pts, a WHO grade 2 cutaneous cGVHD was seen in 2 pts. After a medi-an follow up of 32,2 months (range 5-60), 20 pts are alive (18 in CR, 3 in PD). 4 TRM were documented, 1 for acute respiratory distress syndrome from 2 months from transplant, 1 for aTTP from 18 months from transplant, 2 pts died for liver aGVHD at 3 and 5 months from transplant respectively, 5 pts died for disease recurrence at 10,17,19,21,26 months post-transplant respectively. 1 pts died for Lymphoma non EBV related at 11 months from transplant. Chimerism study showed a full donor sit-uation in 17 pts and a mixed chimerism in 13 pts. In conclusion, NST is feasible and should be considered for high risk lymphoma pts.

**PU-005**

**JAK2V617F MUTATION AS USEFUL MOLECULAR MARKER FOR MYELOPROLIFERATIVE DISORDERS**


A high proportion (>50%) of patients with myeloproliferative disorders (MPD) have constitutional activation of protein tyrosine kinases: JAK2V617F mutation in the JH2 kinase-like domain of JAK2. This mutation leads to deregulation of cytokine activity, and to pathological phagocytosis. A high proportion (>50%) of patients with myeloproliferative disorders (MPD) have constitutional activation of protein tyrosine kinases: JAK2V617F mutation in the JH2 kinase-like domain of JAK2. This mutation leads to deregulation of cytokine activity, and to pathological phagocytosis. Activity of the JAK2V617F mutation in different studies ranges from 65-97% in polycythemia vera, from 41-57% in patients with essential thrombocythemia, and from 28-95% in patients with idiopathic myelofibrosis. In MPD the mutation is heterozygous in most patients and homozygous only in a minor this study, we detected the JAK2V617F mutation by qualitative real-time PCR in peripheral blood samples of patients with myeloproliferative disorders subset. The level in the stem cell hierarchy on which the initiating genetic events of the JAK2V617F mutation occurs, is not known. The mutation has so far been detected in all cells of the myeloid lineage, whereas the potential clonal involvement of the lymphoid lineage is controversial. In this study, we detected the JAK2V617F mutation by real-time quantitative PCR in a large number of patients affected by myeloproliferative disorders. The mutation study could be an useful diagnostic and prognostic marker in the understanding of molecular pathogenesis, that could allow the setting of new therapeutic strategies for the treatment of polycytemia vera and other related disorders.

**PU-006**

**A WERNICKE’S ENCEPHALOPATHY IN A PATIENT WITH ACUTE MYELOID LEUKEMIA**

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A 41 yo women was admitted to our hospital with leukopenia. Acute myeloid leukemia was diagnosed (FAB M1). The patient was eligible for GIMEMA protocol AML 12-one course of HD-ARA-C (6000mg/m^2 every 12 hrs on days 1-3-5-7) was infused intravenously. She developed acute mucositis and anorexia requiring Total Parenteral Nutrition (TPN). On day +29 complete remission was established. At this time the patient experienced visual disturbance (disopia and blurry vision). Symptoms rapidly worsened and included horizontal and vertical nistagmus, ophthalmoplegia and depreses consciousness: she was not oriented to person and had sparse and mildly slurred speech, decreased alertness and attention, perceptive disorientation, poor memory and mild tachycardia. On physical examination, she was afibrile, sleepy but able to be aroused, motor aphasia and general muscular stiffness, reduction of osteotendinous reflexes. Laboratory evaluation revealed no alterations. The results of cerebral fluid studies were negative for leukemic localization and viral encephalitis. The initial cranial CT revealed a low densi-ty area along optical radiation; brain MRI evidenced pathological changes in the medial thalamus, in the 3rd and 4th ventricular floor, in the cerebellar vermis and in the peri-aqueductal gray substance. All these signs were consistent with Wernicke’s encephalopathy (WE). WE is attributable to Thiamina (Vitamin B1) deficiency and is usually associated with alcoholism and malnutrition. Patients who are dependent on TPN without proper replacement of thiamine have also developed WE. Parenteral administration of Thiamina (Benerva 50 mg) was given: she rapidly had marked improvements of mental status, memory loss, slurred speech, and extracocular movements during the next seven days. Because the patient primarily in acute alcoholism, not on TPN, we considered readily a patient with malignancies who are in TPN for long time without vita- min supplementation. Early recognition and rapid treatment with thia-min supplementation is important because the symptoms may improve rapidly and the recovery is complete. With this case report, we bring attention to the importance of proper vitamin supplementation during TPN, to prevent this type of neurological disorders.

**PU-008**

**GRANULOCYTIC PROLIFERATION BY GRANULOCYTE COLONY-STIMULATING FACTOR IN THE LUNG PARENCHYMA IN A PATIENT WITH ACUTE MYELOID LEUKAEMIA**

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A 67-year-old woman with acute myeloid leukaemia FAB subtype M1 developed an antibiotics-resistant fever on day +3 of the induction therapy with MICE (Ara-C, VP-16, and mitoxantrone) regimen. The fever persisted, amphotericin B was added and computed tomography (CT) of the chest was performed. It revealed multiple and bilateral, (non calcified), with irregular margins, pulmonary nodules, suggestive of Bo- gistic lesions. After a few days of therapy fever disappeared; a second CT, eight days after, showed multiple small inflammatory masses with irregular margins and CT halo sign strongly suggestive for opportunis-tic fungal infection. Fibreoptic bronchoscopy to obtain bronchoalveolar lavage specimens was performed. Processing of fluid recovered from...
BAL not proved any pathogen organism. Empirical antifungal therapy administration was continued. Bone marrow aspiration was performed on day +30 after start of chemotherapy; it was hypocellular with 54% myeloblasts. A control chest CT revealed marked size reduction of the multiple bilateral nodules, the largest lesion, in the apical segment of the superior lobe of the right lung, was 1.2 cm in diameter. So the patient was admitted to reinduction therapy with doses of the idarubicina (IDA), Fludarabine (FL), cytarabine (ARA-C), and granulocyte colony-stimulating factor (G-CSF) combination with IDA-FLAG; G-CSF (filgrastim) was started at day 5 at a dose of 5 µg/kg/d until the absolute neutrophil count (ANC) was more than 1,5×10⁹/L (1.500/microL) (for a total 9 days). Complete remission was obtained. All the time of chemotherapy and next weeks itraconazole have been administered to patients; in post-chemotherapy patient didn’t experience fever. Follow-up TC after FLAG-IDA showed persistence of variable-sized nodules and the largest (5 cm in diameter) in right lower lobe. Since radiological imaging was continuously suspected for pulmonary aspergillosis and patient was need to consolidation therapy, an postero-lateral sub-segmental atypical pulmonary resection of the right lower lobe was performed. The histologic examination revealed parenchyma with flogistic nodular infiltrates with small lymphocytes, histiocytes and numerous giant polygonal cell. This description was suggestive extramedullary hematopoiesis after receipt of G-CSF. After surgery the patient received a second course of FLAG-IDA as consolidation. The remains in CR, without any maintenance treatment, for 4 months. In patient with AML, treated with chemotherapy and G-CSF also extramedullary hematopoiesis should be considered in differential diagnosis with myeloma or myeloid leukemic infiltrate when a parenchyma lesion will be identified.

**PU-009**

**CIPROFLOXACIN PLUS AMIKACIN VERSUS IMIPENEM PLUS PIPERACILLIN/TAZOBACTAM: A COMPARATIVE STUDY IN FEBRILE NEUTROPENIA EMPIRICAL THERAPY**

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**Background and objectives.** Hematologic patients are highly susceptible to infectious complications for a number of predisposing factors, mainly prolonged and deep neutropenia. Mortality rate of these infections is not negligible, especially as to Gram-negative sepsis, so prompt broad-spectrum antibiotic therapy should be started before microbiological investigations results. Several approaches to the empirical antimicrobial management of neutropenic patients infections have been planned. Most regimens include either monotherapy or two-three antibiotics combinations. In our clinical trial we compared efficacy and safety in neutropenic oncohaematologic patients of two antibiotic combination regimens: amikacin plus ciprofloxacin (AC) versus imipenem/cilastatin plus piperacillin/tazobactam (ICPT). **Methods.** We performed a comparative, randomized, non placebo-controlled study of 51 hospitalized patients who had neutropenia and fever after chemotherapy for hematologic malignancies. Neutropenia was defined as an absolute neutrophil count (ANC) below 500 per cubic millimiter, fever as a body temperature of 38°C or higher on 2 measurements within 24 hours. All recruited patients had an high risk of infectious complications, specifically, deep (ANC<100/mmc and prolonged (> 7 days) neutropenia with a Multi National Association for Supportive Care in Cancer Risk Index (MASCRC score) over 21, that legitimated an empirical approach of broad-spectrum intra-venous antibiotic therapy. Prophylaxis with acyclovir and fluconazole i.v. as well as granulocytopoietic growth factors use were allowed. During neutropenic febrile phase no serological and microbiological finding (i.e. blood, urine, pharyngeal material cultures, galactomannan test, etc.) was detected (Fever of Unexplained Origin or FUO). At fever onset neutropenic patients were assigned to receive alternatively either amikacin 1.5 g ivid plus ciprofloxacin 400 mg bid or imipenem/cilastatin 1 g tid plus piperacillin/tazobactam 4.5 g qid. If fever persisted at tenth day of anti-bacterial and treatment, the starting antibiotic regimen was shifted to the other (AC to ICPT or inversely). Patients continued treatment until neutropenia resolution, defined as an increase in the ANC at least up to 1,000/microL at the ANC level. At this level, patients with no documented cause of infection could be discharged from antimicrobial therapy if they became persistently afebrile. Fever resolution was considered success criterion of empirical antimicrobial therapy regimen. Treatment was considered to have failed if the regimen didn’t produce defervesce overall after 10 days or if one regimen had to be switched to other. In the latter event, if defervescence was obtained, replacing regimen was esteemed successful. **Results and Safety.** Of 51 patients examined, 26 received the AC regimen, 25 the ICPT. In the AC group FUO was successfully treated in 20 patients with a success percentage of 76.9 percent. In 17 of these fever resolved occurred on AC regimen within 6-7 days in over 90 percent of them. 11 were given a granulocyte-macrophage colony stimulating factor -GMCF- in addition to the assigned treatment Among the six unresponsive patients, three were switched to ICPL scheme (see below), others died before change. FUO resolution was observed in 22 of 25 patients of ICPT regimen. Moreover, 3 of 26 patients initially assigned to AC regimen were switched to ICPT which allowed to obtain defervescence. The overall success percentage in ICPT group was 89.2 percent. 21 patients became afebrile on ANC rise within 5-6 days in over 90 percent. 9 of them also received GMCF. The three unresponsive patients of this group refractory patients died, two before regimen substitution, the remaining just two days after switch to AC scheme Both regimens turned out well tolerated. Adverse effects occurred in 5 of 26 patients treated with AC regimen (19,2 percent) and consisted of mild transient erythema in the area of ciprofloxacin intravenous infusion. There was no need to withdraw antimicrobial treatment. No side effect was observed in patients of ICPT group. **Conclusions** Through above comparative study we found that combination of imipenem/cilastatin and piperacillin/tazobactam was more effective than association amikacin and ciprofloxacin for the empirical management of FUO in chemotherapy-induced neutropenia of high risk patients, with no side effect and fewer death rate. In oncohaematologic patients with febrile neutropenia, the empiric broad-spectrum antibiotic combination of imipenem/cilastatin and piperacillin/tazobactam could be an efficacy and safe therapeutic approach and an useful option of other proposed regimens.

**PU-010**

**EVALUATION OF CARDIOTOXICITY AND FEASIBILITY OF COMP-R CHEMOIMMUNOTHERAPY IN THE TREATMENT OF NON HODGKIN’S LYMOPHMA PATIENTS: A SINGLE CENTRE EXPERIENCE**

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**Background.** Chemo202immunotherapy (CHOP-R) is the treatment of choice for non Hodgkin’s lymphoma. Many patients are elderly and unable to complete the treatment. Not pegylated liposomal doxorubicin (Myocet®) is a preparation of doxorubicin which has improved therapeutic index in comparison to conventional doxorubicin. Myocet® has a better pharmacokinetic profile with much lower cardiotoxicity. **Methods.** In our department between 2002-2006, 19 patients with non Hodgkin’s lymphoma received cheemoimmunotherapy COMP21-R (cyclophosphamide, Myocet®, vincristine and prednisolone plus Rituximab). The patients (14M and SF) with a median age of 65 (range 51-79) received a mean of 5.5 cycles (range 3-8). Four patients were stage I, 6 stage II, 4 stage III and 5 stage IV. According to IPI score, 2 pts were low risk, 3 low-intermediate, 8 intermediate-high and 6 high risk. Cardiological evaluation was made considering left ventricular ejection fraction (LVEF). Evaluation of treatment efficacy was made in 17 patients: 14 (82.3%) obtained a complete remission, 1 (5.8%) a partial remission and 2 (11.7%) not respond to therapy. At diagnosis the median LVEF was 57% (range 60-45%). At the end of therapy the median LEVF was 51% (range 60-45%). One patient presented acute myocardial infarction after first cycle and suspended chemotherapy temporarily. Three pts. died: one for acute myocardial infarction in complete remission of disease and two died for disease. The median observation period was 29 months (range 6-53) and overall survival was 82%. **Conclusions.** We conclude that Myocet® reduces cardiotoxicity and is effective in the treatment of non Hodgkin’s lymphoma patients.
**PU-011**
A REAL TIME QUANTITATIVE PCR FOR THE QUANTIFICATION OF RESIDUAL MALIGNANT CELL IN NON HODGKIN’S LYMPHOMA

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Follicular Lymphoma (FL) is one of the most common B-cell non Hodgkin’s disease. The basic genetic event found in approximately 90% of FL is the t(14;18) translocation, a rearrangement of the BCL-2 proto-oncogene on chromosome 18 with the immunoglobulin heavy-chain on chromosome 14, the majority of breakpoints on chromosome 18 clustered in the major breakpoint cluster region (Mbr) represents 70% of all breakpoints on chromosome 14. This event determines constitutive expression of the antiapoptotic BCL-2 protein. The analysis of t(14;18) translocation could be a useful method for the evaluation of minimal residual disease in patients affected by malignant lymphoma. In our laboratory we are using a method for evaluation of BCL2/IgH+ residual cells in bone marrow (BM) by Real-Time Quantitative PCR. Multivariate analysis demonstrated that the level of BCL2/IgH+ cells in the BM patients affected by FL is an appropriate marker of clinical and molecular response after CHOP and Rituximab treatment. In this prospective the monitoring of BCL2/IgH+ residual cells in patients affected by FL could be helpful for the prognostic evaluation of tumour progression. Moreover the early detection of the persistence of BCL2/IgH+ cells could allow to perform the most adequate therapeutic strategy.

**PU-012**
MESENCHYMAL STEM CELLS FROM BONE MARROW AND MOBILIZED PERIPHERAL BLOOD: DIFFERENCES IN GROWTH KINETICS

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**Introduction.** Mesenchymal stem cells (MSC) are multipotent cells that are considered one of the most promising product for cellular therapy in regenerative medicine. However, cell dose could represent a critical point in clinical applications. The aim of the present study was to compare the growth kinetics of MSC obtained from bone marrow (BM) and mobilized peripheral blood (MPB). Methods. BM and MPB samples were separated by negative lineage-depletion immunoselection (RosetteSep). Selected cells were seeded in multi-well plates at low density (8-10^6 cells, and 0.9-4.6x10^6 cells) in MesenCult Basal Medium with and without growth factors (EGF, PDGF-BB). On reaching confluence, adherent cells were detached by 0.25% trypsin-EDTA treatment and replated for at least 2 passages (P1 and P2). At each passage, surface antigen expression was analyzed by flowcytometry (CD45-APC, CD34-PE, CD105-FITC, CD44-FITC, CD73-PE, CD166-FITC, CD31-PE, HLA-DR FITC). Expanded MSC were analyzed for differentiation capacities by using specific differentiating medium for osteogenic (MesenCult Human Osteogenic Supplement) and adipogenic (MesenCult Human Adipogenic Supplement) differentiation. Results. Following immunoselection 2-8x10^6 cells, and 0.9-4.6x10^6 cells were recovered from BM and MPB samples, respectively. BM cells reached confluence in 3 weeks; immunophenotyping showed negativity for CD45 and CD34 antigens, and positivity > 90% for CD90, CD73, CD105 and CD166 from passage 2.

**Table 1.**

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>CD90/CD73/CD34</th>
<th>CD105/CD73/CD34</th>
<th>CD166/CD73/CD34</th>
</tr>
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<tbody>
<tr>
<td>P1</td>
<td>69%</td>
<td>93%</td>
<td>66%</td>
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<tr>
<td>P2</td>
<td>94%</td>
<td>69%</td>
<td>92%</td>
</tr>
<tr>
<td>P0.9GF</td>
<td>76%</td>
<td>93%</td>
<td>77%</td>
</tr>
<tr>
<td>P0.4GF</td>
<td>92%</td>
<td>75%</td>
<td>95%</td>
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</tbody>
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**Results.** For the contrary, MPB cells reached confluence in 42% of samples with a delay of 4-6 weeks, compared to BM samples. Moreover, immunophenotyping resulted negative for CD34, while 74%, 68% and 30% of cells expressed CD105, CD166 and CD73, respectively. Discussion. We confirm that expansion and differentiation of BM-derived MSC is feasible, reproducible and fairly rapid. On the other hand, expansion of MPB-derived MSC seems more difficult and not reproducible in all cases. Therefore, further studies need to be conducted to find better culture conditions and GF combinations to support MPB-derived MSC expansion.

**PU-013**
HEPATITIS B REACTIVATION IN PATIENTS UNDERGOING AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

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Hepatitis due to reactivation of hepatitis B virus (HBV) is an important cause of liver related disease in patients undergoing autologous hematopoietic cell transplantation. It has been observed in patients positive for hepatitis B surface antigen (HBsAg) and in HBsAg negative patients who had HBV infection in the past (HBsAb and HbcAb positive). We investigated the incidence of hepatitis B reactivation in 20 patients undergoing autologous hematopoietic cell transplantation in our institution between April 2005 and December 2006. Of these 20 patients, 12 were seronegative for HBV, 6 were anti HBs (HBsAb) and 2 were healthy carriers of hepatitis B surface antigen (HBsAg). Prior to transplantation, all patients had normal levels of alanine aminotransferase (ALT), aspartic aminotransferase (AST), alkaline phosphatase and total bilirubin. Liver function tests were carried out thrice weekly during the first 30 days of transplant, once a week during the next 30-60 days, and at 2 to 12 week intervals until their last follow up. The patients were followed for a median of 14 months after transplantation. Hepatitis B reactivation developed in 3 patients after transplantation. One of these patients was HBsAg positive and two were HBsAb positive before transplantation. Hepatitis flare-up occurred at a median of 4-6 months after autologous transplantation. These patients become HBsAg positive, HBeAg and anti Hbc IgM positive. HBV DNA was positive for all three patients. In two patients hepatitis was documented with an elevation of transaminase and bilirubin levels. Lamivudine treatment was started in all these patients. One patient recovered completely after this treatment. Two patients are still treated with lamivudine. Our result indicate that HBV reactivation post autologous hematopoietic cell transplantation is possible not only in HBsAg positive patients, but also in HBsAb and HbsAg positive patients. We suggest that a careful monitoring of HBV-DNA levels is important for prevent hepatic damage caused by HBV reactivation.

**PU-014**
MONOTHERAPY WITH PEGYLATED LIPOSOMAL DOXORUBICIN (PEG-DOXO) IN THE TREATMENT OF PRIMARY CUTANEOUS B CELL LYMPHOMA

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**Introduction.** The therapy of advanced, relapsed or refractory primary cutaneous lymphomas is often unsatisfactory. Recent literature data indicate that the favourable pharmacokinetic, pharmacodynamic and toxicity profiles of pegylated liposomal doxorubicin (Peg-Doxo) can be exploited in these patients; it has been used in cutaneous T-cell lymphomas (CTCL), but hasn’t been evaluated in cutaneous B-cell lymphomas (CBCL) to date. We performed a prospective phase II clinical trial using Peg-Doxo monotherapy in CBCL. Methods. Peg-Doxo (20 mg/m²) was administered every 3-4 weeks in four patients. Results. All patients had multiple generalized nodular skin lesions; the PS was 0 and no one had systemic symptoms. The first was a 38-year-old woman affected by a relapsing primary cutaneous marginal zone B-cell lymphoma (MZBCL) for 25 months, pre-treated with radiotherapy, followed after a recurrence by gemcitabine. With Peg-Doxo she reached a complete response (CR), yet relapsed after 8 months. She was then treated.
with interferon and rituximab, achieving a CR. The second patient, of 55, affected for 35 months by diffuse large B-cell lymphoma (DLBCL), leg type and previously treated with radiotherapy, with a partial response (PR), followed by CR with R-CVP chemotherapy. After a relapse he was treated with 8 infusions of Peg-Doxo, reaching a CR after 2 months, still lasting after 51 months. The third and the fourth were untreated; a 75-year-old man, affected by DBLCB, leg type, obtained a CR after Peg-Doxo, yet relapsed after 11 months. He received four courses of CBVD scheme (peg-doxxo, bleomycin, vinblastine, dacarbazine), then followed by a maintenance with rituximab, achieving a clinical CR; the latter, a 55-year-old man, affected by DLBCL, reached a CR still lasting after 45 months. Peg-Doxo monotherapy was successfully tolerated and no patients decreased or delayed the dose. Discussion. All the patients responded well to the therapy (CR=100%), even when pretreated and/or experiencing the most aggressive forms, like DLBCL, leg type. It is noteworthy that all patients reached a clinical CR in a very short period (median 2.5 months). The treatment was well tolerated and safe. Notwithstanding the small number of patients it emerges that monotherapy with Peg-Doxxo shows a significantly high clinical activity and a worthy that all patients reached a clinical CR in a very short period.

**Table 1. Clinico-pathological characteristics of the CBCL patients.**

| P | Sex | Age | Time | CBCL | Pre | Les | Max | Inf | Res | Tax | Tox | Rel | FUP |
|---|-----|-----|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1 | F | 38 | 23 | MZBCL | R.C. | Nod | 2 | 4 | CR | No | Yes | CR | 3 | 1 |
| 2 | M | 55 | 35 | DBCLC | R.C. | Nod | 2 | 8 | CR | No | No | CR | 51 |
| 3 | M | 75 | 2 | DBLC | None | Nod | 4 | 6 | CR | No | Yes | CR | 11 | 19 |
| 4 | M | 55 | 2 | DBLC | None | Nod | 3 | 6 | CR | No | No | CR | 45 |

P: patient number; Time: months from the diagnosis to Peg-Doxxo; CBCL, MZBCL, DBLC: see text; Pre: therapy pre-Peg-Doxxo; R: radiotherapy; C: chemotherapy i.e. gemcitabine (P1) and immunologic (cyclophosphamide, vincristine, prednisone (P2)); Les: nodules; Max: months for CR; Inf: number of infusions; Res: toxicity; Rel: relapse and time; FUP: follow up and time.

**PU-016**

**OSTEONECROSIS OF THE JAWS IN MULTIPLE MYELOMA PATIENTS TREATED WITH ZOLEDRONIC ACID: A SINGLE-CENTRE EXPERIENCE**

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Bone destruction is a significant cause of morbidity in patients with multiple myeloma. The use of bisphosphonates is well established for the treatment of patients with metastatic bone disease. It has been demonstrated that bisphosphonate-based supportive therapy (pamidronate or zoledronate) reduces skeletal events (onset or progression of osteolytic lesions) both in patients with multiple myeloma and in cancer patients with bone metastasis. Bisphosphonates are generally well tolerated and associated with minimal adverse effects: fever, renal function impairment, myalgias and hypocalcemia. In recent years, several cases of jaw bone necrosis associated with long-term use of bisphosphonates have been reported. The estimated incidence varies from 1.8% to 12.8% (Hematology 2006). The pathogenesis of this complication is unknown; however, several predisposing factors have been identified: poor oral hygiene, periodontal disease, dentoalveolar surgery, corticosteroid therapy, immune-compromised state predisposing to increased risk of infection. We performed a retrospective study on osteonecrosis of the jaws in 38 multiple myeloma patients with a history of chronic zoledronic acid therapy. Between January 2004 and December 2006 we observed four patients with osteonecrosis of the jaws (10.5%). Diagnosis was radiological and clinical. CT scan confirmed the presence of an osteolytic area with signs of periosteal reaction. Biopsies from the necrotic lesions revealed no metastatic disease. Microbiology showed actinomycetes and mixed bacteria. The characteristic of the patients were the following: median age = 65 years (46-81), M/F = 2/2, IgG/IgA = 3/1 kappa/lambda = 3/1. Steroid use = 3 patients. Thalidomide use = 3 patients. Autologous stem cell transplantation = 1 patient. Previous dental extraction = 2 patients. Median time of exposure to zoledronic acid = 10 months (8-13). Amino-bisphosphonates exert several anti-tumor effects, including induction of tumor cell apoptosis, inhibition of tumor cell adhesion to the extracellular matrix, and inhibition of tumor invasion. Zoledronic acid also have antiangiogenesis properties and can activate gamma-delta T cells. It has resulted in a statistically significant reduction in skeletal complication. We have initiated the following guidelines in an effort to ameliorate the incidence of jaw bone necrosis. Patients have a screening dental examination and an appropriate radiographic study before the administration of zoledronic acid. They are encouraged to practice good dental hygiene and see a dentist promptly if oral or dental symptoms appear. In addition, zoledronic acid are held for a period of 3 months prior to invasive dental procedures to allow for the osteoclastic recovery. Following the dental procedure we re-introduce bisphosphonates only after the healing process is complete.

**Introduction.** The emocomponent donation, by MCC procedure, is able to assure optimal transfusional products, a good donor compliance and an relatively low frequency of A.E. Materials and Methods. From January to June 2005, in the SIMT at O.V.E. of Catania, 845 procedures in MCC have been executed. They were divided in: CRC-PLT n°29 (5.3%), PLT-plasma 364 (67%), double PLT 150 (27.2%). The donors were 465 men (79.6%), and 108 women (%), median age was 42 years (min20; max65), middle weight was 78.7 Kg (min 47; max 130), middle height was 171 cm (min 148; max 193). After an secured medical visit, the donor were chosen referencing to emocomotoxicometric parameters. Donors have expressed their consent by signature. Refering to the venous access, our equipe have used the following cellular separators: Cobe Spectra (Colorado USA) n°71 proc. (12.9%), Haemonetics plus (Massachusetts USA) n°117 proc. (21.3%), Fresenius Haemocare Com.tec (Germany) n°357 proc. (61.8%). Results. In each apheatic procedure we have valued: the during, the ACD quanty used and the presence of A.E. As a result of our study, the complexive A.E. were 93, so distribueted: addominal pain (1%), parestesia (53.8%), cefalea (1%), trumble (52.5%), vomiting (1%) and nausea (5.4%). The procedure was never interrupted. The procedure was continued after the donor's consense, when the symptom was resolved. The middle time was 71 minutes (min 26: max 200), the ACD used was 276 ml (min101;max556). Refering to the celluar separator used, the A.E. were divided obtening: Cobe Spectra we got 14 A.E. in 71 procedures (19.7%), Haemonetics plus we got 12 A.E. in 117 procedures (10.2%) and Fresenius haemocare we got 71 A.E. in 327 procedures (21.1%). In addition, the A.E. relative to ACD intolerance were 12% for Cobe spectra, while 11.8% for Fresenius and 10% for Haemonetics. In the end, the donors were divided in 2 groups, refering to their age: over 45 and under 45. In our study the A.E. verified in under 45 group were more frequent than those verified in over 45 group: 63% vs 57%. Conclusion: this work got as a result that the A.E. rate was 17.96%. We underline the fact that in under 45 donors the E.A. was most seen (over 45 group: 12.8% vs 6%). This result depends on the fact that the main part of under 45 donors are at their first experience. The MCC procedure is good tolerated independently from the cellular separator used. Infact, in our study beyond the vaso-vagal reaction and for those relative to the ACD intolerance, there aren't series A.E. that haven't permitted to continue the procedure.

**PU-017**

**HEMOPOIETIC STEM CELLS HARVEST AND THE COLLECTION EFFICIENCY (C.E.)**

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Introduction. As recently confirmed by EBMT more than 60% of emopoietic bone transplants are made throught periferic blood of stem cells (PBSC). At the SIMT Vittorio Emanuele hospital of Catania, 8 procedures of PBSC were done during the 2006, to familiar donor HLA sib-
ling. The object of our study was the C.E. of two different cellular separ-
ators (C.S.): Cobe Spectra (USA Colorado, Massachussets) and Frese-
nius Com. (TEC, Germany). Material and Methods. Cobe Spectra than
was used for 4 PBSC collections and the same number for Fresenius Com.
TEC. Patients were 5 men and one woman, middle age was 37 years
(27min; 61max), a middle weight of 75 kg (min 70; max 87), a middle
height of 168 cm (min 162; max 181) and their blood volume was 4797
ml (min 4538; max 5378). Collection timing was when the CD34+ in
peripheral blood was superior to 20 microl, while the collection was in-
terrupted when the concentration of CD34+ was equal or superior to
5×10^4/kg recipient’s body weight. The middle of CD34+ in peripheral
blood in donors was 63microl (min 20.2; max 155), 4 patients under mobil-
ization with growth factors (G-CSF) glcosilated were treated to apheresis
in the 5th day, while 2 (not good mobilization) were underlied to 2
apheresic collection, during the 5th and 6th day. The middle blood vol-
ume processed was 11489 mL (min 10000; max 13591). Result: We have
obtained the seguent results in the harvest: collection volume 235 ml
(min 186; max 270), median number of CD34+ cells collected was 11
per leukapheresis and total number of CD34+ cells in patients weighting
< or > 60 kg or < 70 > kg. Actually, 34 patients received a single transplan-
tation and 47 a tandem transplantation, with rapid engraftment.

**PU-018**

**IFOSFAMIDE, GEMCITABINE, VINORELBINE, PREDNISOLONE (IGEV) AND FIXED DOSE OF
LENORAGRISTIM: AN EFFECTIVE MOBILIZATION REGIMEN IN PRETREATED Hodgkin’s
LYMPHOMA PATIENTS**

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Introduction. In this study we explored the efficacy of IGEV regimen
combined with fixed dose of lenograstim (263 μg day) to mobilize periph-
eral blood stem cells (PBSCs) in resistant/residual Hodgkin’s lymphoma
(HL). Methods. Ninety patients were treated prospectively with a salvage
regimen consisting of ifosfamide 2000 mg/m2 on days 1 to 4, gemcitabine
800 mg/m2 on days 1 and 4, vinorelbine 20 mg/m2 on day 1, and
prednisolone 100 mg on days 1 to 4 (IGEV). A fixed dose of lenograstim (268
μg day) was given from day 7 to day 12 of each course or up to apheresis
during the mobilizing phase. Results. Leukapheresis was performed after
the first, second, and forth cycles of chemotherapy in four, seven, seven-

cases, and eight cases, respectively. The median total CD34+ cell/µL peak, CFU-GM and white blood cells (WBC) for all individual set
of collection was 85/µL, 12×10^6/Kg, and 2070/00/µL, respectively. In all cases,
stem cell harvesting started after a median of 15 days from the first day
of IGEV chemotherapy (range 10-17). An adequate CD34+ cells (over 3×10^6
or 6×10^6 CD34+ cells/kg according to single or tandem high-dose
chemotherapy procedures) collection was achieved in 89 out of 90 (98,7%)
mobilized patients, the only failure pooling 2,5x10^6 CD34+ cells/kg.
The median of CD34+ cells collected was 11×10^6; (range 5.1x10^5-23.9x10^6/kg)
with a median of one (range 1-3) leukaapheresis for patients eligible
for single high dose treatment, and 10x10^6/kg (range 6-
22,0x10^6/kg) with a median of 2 (range 1-3) leukaapheresis for candidates
to tandem transplant, respectively. Overall, the target yields were reached in
71,48% and 49,09% of cases after the first apheresis procedure and in
further 17,14% and 48,64%, after the second apheresis, respectively. There
were no significant differences in the total number of CD34+ cells per
per leukapheresis and total number of CD34+ cells in patients weighting
< or > 60 kg or < 70 > kg. Actually, 34 patients received a single transplan-
tation and 47 a tandem transplantation, with rapid engraftment. Conclu-
sions. These results confirm that IGEV regimen with lenograstim support

can be successfully and safely used to mobilize PBSCs.

**PU-019**

**THE RARE COEXISTENCE OF CLASSICAL Hodgkin Lymphoma IN A NODAL MARGINAL
ZONE B-LYMPHOCYTE PROLIFERATION**

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In rare cases of marginal zone B cell Lymphoma, large cells morpho-
logically similar to or indistinguishable from Hodgkin/Reed-Stemberg
(HRS) cells of Hodgkin’s disease can be found in a background of oth-
ewise typical marginal zone B-cells lymphoma. The biologic relation-
ship of these two malignant neoplasm often is unclear. We describe a
case of a 71 year old woman affected by marginal zone B-cell nodal
lymphoma involving axillary, retroperitoneal and inguinal sites. In
August 2003 the study of lymph node biopsy was consistent with the
reported diagnosis of marginal zone B cell lymphoma (immunopheno-
typing with CD20, CD19, CD22, CD79a positive; CD5, CD10 and
CD22 negative) but an atypical large cells population was present, with
and without morphology features of HRS. The patient was treated with
six cycles of combination chemotherapy according with COP regimen.
Complete clinical and haematological remission was archived in Febru-
ary 2004. At the restaging in September 2005 a PET-CT scan revealed
the existence of retroperitoneal lymphadenopaties. The patient was
-treated with a second line therapy according with R-CHOP regimen (6
cycles). After a second complete clinical and haematological remission,
at December 2007 restaging we detected a right later-cervical lymph-
adenopaties and omolateral swelling of front-temporal region. In
this case the lymph node biopsy revealed a disappearance of any clon-
ally light chain restriction of B cells population but checked an unexpect-
ed Hodgkin lymphoma characterized by mixed cellularity (immunophe-
notyping with intense and diffuse positivity for CD30, MUM1 and
CD15). We may speculate that the presence HRS like cells have an
increased risk develop to an HRS-like cell clone of HD, perhaps upon
acquiring additional transforming event. We did not study the rearrange-
ment involved in different VH and JH genes, eventually proving the
same origin of two type of lymphomas diseases. Tumour biological fea-
tures common to both HD and NHL may indicate a similar cellular ori-
igin, regardless of the time interval between the diagnoses. This report
confirm the rare coexistence of classical Hodgkin lymphoma in a nodal
marginal zone B-lymphocytes proliferation and the need of a more cor-
rect therapeutic strategy.

**PU-020**

**SERUM ALKALINE PHOSPHATASE LEVEL AS A PROGNOSTIC FACTOR IN Hodgkin’s
DISEASE**

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The efficacy of the currently available treatments for Hodgkin’s dis-
 ease (HD) has led to a substantial modification in the prognosis of this
disease; nevertheless there is still a group of patients that cannot be
cured with conventional treatments and who will be candidates for
alternative therapy. Medical records of all patients with HD treated in
our institution between June 2001 and November 2006 were reviewed.
We treated 47 patients (28F and 19M; median age: 52 years, r.: 19-72
years, stage II-III: 36pcs, stage III-IV: 11pcs) with 4-6 cycles of ABVD reg-

ding in all patients we have monitored serum alkaline phosphatase
(SAP) at diagnosis during and after treatment chemotherapy. In 10 out
of 47 patients we found an increment of serum alkaline phosphate lev-
el. During treatment chemotherapy six out of these ten patients (4F and
2 M, two stage I-II and four stage III-IV) showed stability disease (SD)
and SAP level over 230 units/l, while 4 patients (2F and 2M, stage III-IV)
showed progression of disease (PD) and SAP level over 380 units/l. The
due PD patients were treated by four cycles of IGEV regimen and autol-
sal bone marrow transplantation (AuBMT) but two of them dead for
progression disease. The six SD patients showed resistance to chemotherap
ey and were treated by three-four cycles of IGEV regimen and
AuBMT. All these patients achieved a complete remission. We con-
clude that persistent elevated SAP level may be considered a risk factor

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in HD patients both in early and advanced stage. By monitoring SAP level during treatment chemotherapy we can individuate a subset of patients with worse prognosis who will be candidates for more aggressive therapeutic options.

**PU-021**

**TRANSFORMATION OF FOLLICULAR LYMPHOMA ON MAINTENANCE RITUXIMAB TO HODGKIN DISEASE:A CASE REPORT**

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**Background.** Although transformed lymphoma (FL) has a reported incidence of 10-70% among patients with follicular lymphoma (FL), this complication in the form of Hodgkin Disease (HD), as recently observed by us, represents an exceptionally rare event, which poses some therapeutic concerns. Indeed HD, when occurs as transformation of lymphoproliferative disorders, is generally provided of high aggressiveness and portrays in most cases a poor prognosis with reported survivals of few months. 

**Case report.** A 67-year-old male patient was diagnosed as having a stage IIIA (grade 2) FL in August 2005. He was enrolled in a clinical trial and received four cycles of FND (fludarabine, mitoxantrone and dexamethasone) which resulted in a complete response (CR). Therefore, he received 4 weekly doses of rituximab (375 mg/m²) as consolidation treatment and then the same dose every two months as maintenance therapy. On October 2006, having the patient received the last rituximab dose two months before, he presented with general malaise, sweating and weight loss. The physical and radiological evaluations showed cervical, para-aortic, enlarged lymph nodes. A comprehensive laboratory work up revealed mild normochromic anaemia and elevated levels of lactate dehydrogenase and erythrocyte sedimentation rate. A cervical node was removed and the histological diagnosis of HD, lymphocyte depletion subtype, was made (Figure 1). The patient was treated with two cycles of IEV (ifosfamide, epirubicin and etoposide) achieving a CR and then consolidated by an autologous stem cells transplantation (ASCT). To date, three months after the ASCT, he is well and in CR.

**Figure 1.**

Conclusions. The present case is of an aggressive HD with B symptoms developed in a patient with relapsed FL under maintenance therapy with rituximab. Given that no study in order to establish a clonal relationship of the two disease entities were performed, we are able to answer the question, in our patient, as to whether FL and HD originated from the same B cell or from unrelated B cells, although recent studies have suggested a common pathogenic background for HD and FL; indeed, it has shown that Reed-Sternberg cells are also B-cell derivatives. To date, there are no reliable markers of risk for transformation or factors predictive of survival. However, the prognosis for transformed lymphoma is generally poor, with most patients surviving only a few months. Therefore, the clinical means associated with the development of HD were interpreted by us as a highly aggressive neoplastic progression, for which we treated the patient with an intensive high dose chemotherapy and ASCT.

**PU-022**

**SJOGREN’S SYNDROME AND PRIMARY BLADDER LYMPHOMA**

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Primary malignant lymphoma involving urinary bladder is a very rare disease with fewer than 100 cases reported in the literature. We describe a case of primary bladder lymphoma of the MALT-type in a 69-year-old woman with clinical history of HCV infection and Sjögren’s syndrome treated in 1990 with alfa-interferon for ten months, followed by low doses of steroid. In August 2006 the patient reported hematuria, dysuria and urgency; no other symptoms as weight loss, fever, chills or night sweat were present. On physical examination lymphadenopathy or hepatosplenomegaly were excluded. At cistoscopy a nodular mass involving the bladder base and trigone, without urethral obstruction, was observed. A transurethreal biopsy showed a lymphoid infiltration of small cells with morphologic and immunophenotypic features of low-grade non Hodgkin B cell lymphoma of MALT. Bone marrow biopsy was negative. Abdominal MRI and WB FDG-PET confirmed the presence of a large pelvic mass. She was treated with chlorambucil, steroids and rituximab. A cistoscopy performed after 4 cycles showed a residual mass with minimal lymphoid infiltrate. Considering the near complete remission also confirmed by second FDG-PET, the patient completed the treatment with 2 cycles of chemoimmunotherapy; no radiotherapy was performed. There are some considerations to draw in this case. Patients with Sjögren’s syndrome and HCV infection have an increased risk of developing non Hodgkin’s lymphoma: the previous treatment, low serum complement and reduction of CD4+/CD8+ ratio could play a role. MALT lymphoma is more frequently localized in the stomach as a result of specific immune reactions to Helicobacter pylori; the rare cases of primary bladder lymphomas in the literature were MALT type associated with a very good prognosis. In our case the option to treat with chlorambucil and anti CD20 was driven by the concomitant presence of the associated Sjögren’s disease. FDG-PET scans demonstrated his utility both in the staging and in the control of the response to the treatment strategy.

**PU-023**

**EXTRACORPOREAL PHOTO CHEMOTHERAPY FOR TREATMENT OF CHRONIC GRAFT VERSUS HOST DISEASE: THE CATHOLIC UNIVERSITY EXPERIENCE**


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Introduction. Chronic graft-versus-host disease (cGvHD) is the most common and severe complication among patients surviving >100 days after allogeneic stem cell transplantation (ASCT). When drugs are not effective other approaches are needed to control the disease and to reduce drug exposure. Since September 2005 at our Institution we began to treat refractory cGVHD with extracorporeal photochemotherapy (ECP). Methods. We treated 5 patients: 2 male and 3 female with a median of age 52 years (41-60). They underwent ASCT from HLA identical donor (peripheral blood or bone marrow) for hematological malignancies (AML, CML). All patients developed extensive cutaneous cGVHD (osteointestinal involvement in one patient, liver and gut involvement in another one) and received oral steroid therapy alone or in association with tacrolimus and/or micophenolate and cyclosporine A. Because of lack of control cGVHD despite drug therapy and/or the onset of intolerable side effects, patients were submitted to ECP on two consecutive days every month until clinical response was achieved. Results: We performed a total of 34 ECP procedures, with a median number of 6 (range 2-14) per each patient. Twenty-four collection procedures were carried out using continuous flow cell separator (Fresenius COM.TEC, Cobe Spectra) and 6 using discontinuous flow cell separator (Haemonetics MCS+) in one patient with difficult venous peripheral access. The median patient total blood volume processed per procedure was 1.5 in a median time of 183 minutes. The number of total nucleated cells collected per
procedure was 11.45×10⁹, with a mononuclear cell percentage of 80.3% and a hematocrit of 2.7% in a volume of 162 mL. After addition of 8 methoxypsoralen in a continuous solution, UV-A irradiation was performed using PFT System (Med-Tech Solutions, GmbH) with a median of 23.19 Joules in a median time of 16 minutes. Photo-activated cells infusion was well tolerated and side-effects (chills, fever, headache) occurred after one procedure. Discussion. All patients improved and 4 out of 5 patients, after a median of 2 procedures, became independent from steroids and/or reduced immunosuppressive drugs. Procedures performed with discontinuous flow cell separators resulted in larger volume products with smaller cell yields, also requiring a longer irradiation time. In our experience ECP is a safe and effective procedure to control GVHD and/or to reduce drug exposure. Additional studies will establish the role of cell dose.

**PU-024**

**BORTEZOMIB IN RELAPSED OR REFRACTORY MYELOMA PATIENTS**

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Introduction. We analyzed the efficacy, safety, and cost effective of Bortezomib in refractory or relapsed MM patients. Methods. We reviewed 19 patients with relapsed or refractory multiple myeloma treated with Bortezomib at our institution between October 2003 and March 2007. 8/19 pts (42%) received Bortezomib as second line of therapy, post stem cell transplant (group A) and 58% received more than 2 lines of therapy (group B). Patients received Bortezomib 1.3 mg/m² as an intravenous bolus twice a week for 2 weeks, on days 1, 4, 8, and 11, in a 21 day cycle with addition of Dexamethasone 20 mg on the day of and the day after Bortezomib for up to 4 cycles. PFS, TTP and DOR were evaluated. Ducu (LeuKemia 2006; 20, 1467-1473). Results. The mean age was 64 yrs (42.75). Only 8 pts (42%) completed 4 cycles of therapy, while 7 pts (36%) discontinued Bortezomib for toxicity and 4 pts (21%) had a progression while undergoing therapy. The overall response rate was 38.5%; 6 pts (31.5%) achieved PR, 1 pt nCR (5%) sec. Blède criteria. No patients achieved CR. No difference in the response rate was observed between group A and B. The median follow up was 5.3 months; 1 year PFS was 34%; the median TTP was 6.8 months and median DOR was 8.1 months. Grade 3 non Hematological toxicities was observed in 8 patients (42%) and included: Diarrhea (5 patients); Cardiac failure (1); Peripheral neuropathy (1); Pneumonia (1); 5 patients had grade 3 thrombocytopenia. No grade 4 toxicity was observed. 70% patients showing grade 3 adverse events, were older than 65 years. 1 patient aged 75 yrs died of ischemic bowel disease, related to treatment. Conclusion. In our study Bortezomib showed a moderate activity, mostly PR. The DOR is similar to the one reported by conventional salvage chemotherapy, related to treatment with Bortezomib. Conclusions. In our study Bortezomib showed a moderate activity, mostly PR. The DOR is similar to the one reported by conventional salvage chemotherapy, however the latter is less expensive and toxic. We believe that Bortezomib is not cost effective agent in relapsed, elderly MM patients. Moreover we believe more studies are needed to warrant the role of Bortezomib in a younger subset of patients with earlier disease.

**PU-025**

**SIMULTANEOUS DIAGNOSIS OF POLYCYTHEMIA VERA AND CHRONIC LYMPHOCYTIC LEUKAEMIA**

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Simultaneous presentation of polycythemia vera (PV) and chronic lymphocytic leukemia (CLL) is reported in the literature. Few cases of PV and CLL have been reported, in where the occurrence of the two disorders was simultaneous, or sequential but spontaneous. We describe a patient aged 75 yrs died of ischemic bowel disease, related to treatment with Bortezomib. No patients achieved CR. No difference in the response rate was observed. 70% patients showing grade 3 adverse events, were older than 65 years. 1 year PFS was 34%; the median TTP was 6.8 months and median DOR was 8.1 months. Grade 3 non Hematological toxicities was observed in 8 patients (42%) and included: Diarrhea (5 patients); Cardiac failure (1); Peripheral neuropathy (1); Pneumonia (1); 5 patients had grade 3 thrombocytopenia. No grade 4 toxicity was observed. 70% patients showing grade 3 adverse events, were older than 65 years. 1 patient aged 75 yrs died of ischemic bowel disease, related to treatment. Conclusion. In our study Bortezomib showed a moderate activity, mostly PR. The DOR is similar to the one reported by conventional salvage chemotherapy, related to treatment with Bortezomib. Conclusions. In our study Bortezomib showed a moderate activity, mostly PR. The DOR is similar to the one reported by conventional salvage chemotherapy, however the latter is less expensive and toxic. We believe that Bortezomib is not cost effective agent in relapsed, elderly MM patients. Moreover we believe more studies are needed to warrant the role of Bortezomib in a younger subset of patients with earlier disease.

An increased number of erythroid cells and an infiltration of lymphocytes 40 per cent were detected. These cells were CD19⁺ CD20⁺ CD23⁺ CD42⁺. The cytogenetic analysis of the bone marrow revealed a normal karyotype, while the polymerase chain reaction did not show the presence of bcr/abl fusion gene or mutations V617F of the jak2 gene. It was made diagnosis respectively of polycythemia vera, according Polycytemia Vera Study Group criteria and chronic lymphocytic leukaemia Rai stage 2 according to criteria of NCI. The patient was treated with intermittent administration of hydroxyurea without treatment for chronic lymphocytic leukaemia for two years, with mild course of the diseases. Simultaneous presentation of chronic lymphocytic leukaemia and polycythemia vera is rarely reported in a previously untreated patient. Usually this association in the same patient occur only after cytotoxic therapy for PV. The course was remarkably mild with almost no treatment, suggesting control of each disease by the other. Our patient was treated with intermittent administration of hydroxyurea only. Our case suggests the hypothesis that these two disorders could originate from trasformation of a stem cell which successively differentiates in two subclones bearing respectively myeloproliferative and lymphoproliferative disease.

**References**


**PU-026**

**BLOOD DOPING: WHAT A STRATEGY TO DETER IN SPORT?**


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Blood doping consists to utilize illegal methods or substances administered for non medical reasons to athletes to improve the blood oxygen carrying capacity and in turn performance especially in endurance sports (cycling, cross-country skiing, long distance race). In the 1990s blood doping was achieved by blood transfusion from a donor (allogeneous) or from self (autologous). Since the mid 1980s erythropoiesis-stimulating substances, in particular recombinant human erythropoietin (rHuEpo), have been the most used doping techniques. Although blood doping with rHuEpo is recognised in professional cycling and cross-country skiing, several observations by CONI (Comitato Olimpico Nazionale Italiano), indicate that the rHuEpo-users is increasing. This other discipline is not now involved in professional athletes too. The implementation of reliable direct and indirect tests to identify rHuEpo-users, prompt some athletes to experiment new original and unpredictable blood doping substances (cobalt chloride) or strategy (altritracer 200). Consequently detection of these practices is still debating. Several research groups have attempted to improve the strategy to deter blood doping in sports, including markers of accelerated erythropoiesis which if used simultaneously are potentially effective for identifying current (On-model) or recent (OFF-model) rHuEpo-users. Since it is recognised a considerable variability in haematologic parameters from subject to subject, this event implies the need for the use of subject-specific reference range that could distinguish between physiological and abnormal variability. Thus a sequential determination of peripheral blood parameters, may became the opportune method either to define the individual haematologic profile and to early detect a doping substances. In the light of this, according to FIP /Federazione Italiana Pallacanestro and CONI we are activating a prospective study in the aim to determine the haematologic profile in young non professional athletes, through sequential evaluations of haematologic parameters (Hb, RBC, Hct, MCV, MCHC, MCH, % Hypo, serum iron, serum ferritin, transfaring saturation, bilirubin, LDH), at beginning and during -twice year- their own sport activity.
PU-027
LONG TERM SURVIVAL OF MYELODISPLASIA-DERIVED ACUTE MYELOBLASTIC LEUKAEMIA WITH ONLY IDA-VP16 ARAC INDUCTION AND ONE IDA-ARAC CONSOLIDATION COURSE FOLLOWED BY 6-MERCAPTOPURINE MAINTENANCE. A CASE REPORT
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Introduction. Acute Myeloblastic Leukaemia secondary to myelodysplasia (AML-s) presents a worse outcome and the 5 years overall survival is less than 5% for patients not submitted to allogenic haematopoietic stem cell transplantation. Hereinafter we describe a rare case of a long term survival of a patient with this kind of AML, who was only treated with two Idarubicin-Cytarabine courses and subsequently with a maintenance of 6-mercaptopurine 50 mg/day for two years. Case report. A 22 years old man was treated in our department for acute myeloblastic leukaemia in November 2003. Adverse prognostic factors was the evolution of a previous myelodysplasia (a refractory anaemia with excess of blasts) which was diagnosed six months before. A conventional induction chemotherapy with Idarubicin-Etoposide-Cytarabine (54+7) course led to the complete remission, but it was complicated by an Aspergillus Fumigatus pneumonia. A consolidation course with Idarubicin-Cytarabine (2+7) was performed, but a life threatening situation occurred with the development of a multi-focal fungal pneumonia, a contemporary Enterococcus bacteraemia and cardiac failure. Systemic antifungal treatment, large spectrum antibiotics and supportive cares led to a progressive improvement of the clinical conditions, but the previous infective complications contraindicated further chemotherapy. Therefore the patient started a maintenance treatment with 6-mercaptopurine 50 mg/day. This treatment was continued for 2 years, then it was stopped in complete hematologic remission. Actually, 42 months after the onset of the leukaemia, the patient is still in complete remission enjoying a good status and a normal life. Discussion. Favourable evolution of AML-s is an extremely rare event also in presence of intensive treatment, except for allogenic haematopoetic stem cell transplantation. The patient described above was necessarily sub-treated for the occurrence of infective complications, but a long term survival was obtained. We deem that the description of these cases and further study about gene assessment can be useful to select weather there is a subgroup of patients with favourable outcome within the so called “having bad prognosis” categories.

PU-028
PREDONPOSIT AUTOLGOUS BLOOD DONATION (PABD): SURE PROEDURE RESERVED TO FEW PEOPLE EXPERIENCE OF A SINGLE CENTER
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Introduction. The blood lost associated to a lot of programmed surgery, is very elevated specially in old patients; that’s why it needs autologous and onomologous blood transfusion. Materials and Methods. At the SIMT of the Vittorio Emanuele hospital of Catania, during the 2005, 143 patients were underlyed a of (PABD). They were 73 men and 70 women, middle age was 61.5 years (min 19; max 80) and they were affected by different pathologies which needed a surgery correction. Before the PABD, patients were treated with a specific anamnesis to the medical visit. 81patients (56.7%) have executed one PABD, while 62 patients (43.3%) have executed two PABD. The analysis of hemoglobin value, at the first donation was of 14.1 gr/dl (min 12.0; max 17.0), while at the second donation was of 15.2 gr/dl (min 11.3; max 15.3; ds 2.9). Totally, have executed 206 PABD, so distributed referring to the operative unit: orthopedy OVE 94, orthopedy other 35, urology 35, hematology 21, ginecology 7; cardiac surgery and vascular surgery 14. None of patients has presented adverse event during the procedure. Discussion. Occurred analysis of the blood register during the 2005, has proved that over 143 patients treated to PABD only 94 (65.5%) had to do an autologous blood transfusion; in fact only 126 units of autologous blood (61% of units) have been transfused. In addition, we have also noticed how the 12% of patients, who were never treated with autotransfusion (because the autologous units have achieved the for expairs), were after transfused with homologiues units. In the end, we have compared the number of blood units transfusion by homolog transfusion, during the 2005, to the units of orthopedy and urology to the Vittorio Emanuele hospital, with the autologous units transfusion to the same operative units. The obtained result are in the following table:

Table 1. Clinico-pathological characteristics of the CBCL patients.

<table>
<thead>
<tr>
<th>ORTHOPEDY</th>
<th>UROLOGY</th>
<th>TOTAL</th>
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<tbody>
<tr>
<td>94</td>
<td>45</td>
<td>253</td>
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<tr>
<td>35</td>
<td>26</td>
<td>66</td>
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<tr>
<td>129</td>
<td>71</td>
<td>339</td>
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Conclusions. It’s evident that only a small rate of patients benefit of this treatment. In fact, referring to the units of orthopedy and urology during the 2005, 339 omologous units of GRC were transfused including patients who were not treated with autodonation. While only 71GRC units were transfused in predeposit. The result of a specifies clinic valuation of patients is that the autotransfusion by predeposit in patients treated with elective surgery proves to be still an effective and safe procedure, without adverse events verified.

PU-029
SUCCESSFUL TREATMENT OF INTESTINAL ACUTE REFRACTORY GVHD WITH INTENSIFIED EXTRACORPOREAL PHOTOCHEMOTHERAPY IN ASSOCIATION WITH IMMUNOSUPPRESSIVE THERAPY
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Background. graft-versus-host disease (GVHD) is the major cause of mortality and morbility after hematopoietic stem cell transplantation (HSCT). Conventional treatments are sometime ineffective and new therapeutic approaches for the control of GVHD need. Extracorporeal photochemotherapy (ECP) is based on the infusion of autologous blood mononuclear cells collected by apheresis, incubated with psoralen (8-MOP) and irradiated with UVA. ECP has demonstrated efficacy in selected T-cell diseases, including chronic extensive GVHD. Actually literature reports only few cases of ECP treatment in patients affected by acute GVHD with visceral involvement. Case report. We reported a thirty-two women submitted to unrelated HSCT for Philadelphia-positive acute lymphoblastic leukemia, who developed an intestinal acute GVHD after HSCT. Conditioning regimen was TBI-Cyclophosphamide-ATG. GVHD prophylaxis consisted in methotrexate and cyclosporine, but for cerebellar toxicity cyclosporine was substituted with mycophenolate-mofetil (MMF). Molecular remission and full-donor chimerism was obtained 45 days after HSCT. Signs and symptoms of acute GVHD with gut involvement appread in 85th day after HSCT. A colo-rectal endoscopy confirmed the diagnosis and steroid treatment (6-metil-prednisolone 2 mg/kg of body weigh) was promptly started, without significant response. Therefore, we associated immunosuppression treatment with tacrolimus (92 day after HSCT) obtaining a partial response. We decid-ed to submit the patient to intensified ECP treatment starting with two consecutive days (one cycle) weekly for four weeks. After 2 cycles of ECP, the gut symptoms rapidly improved, and 119 day after HSCT endoscopic and histologic re-evaluation was negative for GVHD. After 5 cycles once a week, ECP procedures were performed every 2 weeks for a total of 18 ECP procedures were performed, last in 210th day after HSCT. ECP was well tolerated without any significant side effect. Actually, 1 year after HSCT, GVHD appears controlled and concomitant immunosuppressive treatment with steroid, tacrolimus and MMF has been reduced. Conclusions. Our case report suggests that intensified ECP is safe and effective as adjunctive therapy to pharmacological immunosuppression, for the treatment of severe acute refractory GVHD, also with visceral involvement. A more extensive employ of this procedure could confirm our impression.
PU-030
OSTEONECROSIS OF THE JAW AND BISPHOSPHONATES IN MELIOMA PATIENTS: A SINGE CENTRE EXPERIENCE
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Bisphosphonates (bp) are widely used in the management of metastatic bone disease and hypercalcemia in cancer patients. Pamidronate and zoledronic are anti-angiogenic, antitumor and inhibitor osteoclast agents used in the supportive therapy of multiple myeloma, reducing the severity of myeloma osteopathy and improving the survival in patients exposed to prolonged administration. However in the last few years has been note increasing incidence of osteonecrosis of the jaw in these patients. We report 10 cases of this rare adverse event occurred in years 2000-2005 in 10 myeloma patients followed in our Haematology Unit. Patients’ mean age at diagnosis was 72 years (range 61-78), 4 males and 6 females. The presentations simulate dental abscesses, tooth aches, and osteomyelitis (one case). The jaw osteonecrosis was diagnosed by a maxillofacial surgeon with the support of panoramic x-ray and TC scan. Biopsy of lesions showed no evidence of plasma cell localization or other metastatic disease, but necrotic bone and bacterial infection only. Bone damage was in the mandible (7/10), in maxilla (3/10) or in both (2/10). Tooth extraction preceded the pathologic event in eight cases, but in two, both males, exposed jawbone developed spontaneously. In two cases, both females, bp (first pamidronate and then zoledronate) were the only drug administered, all the other patients have been treated with several regiments of chemotherapy but only two were treated again, no one had received local radiotherapy in past. All patients received monthly acido zoledronate; mean number of administration was 27 (range 40-21), 6 patients were initially treated with pamidronate for 25 mean courses (range 10-44). The two patients untreated with chemotherapy for disease in stage I received pamidronate for 14 and 10 courses and zoledronic acid for 24 and 40 courses respectively. In all patients bp infusion were stopped and started antibiotic therapy and debridement of oral cavity. Somebody required surgical procedures to remove the involved bone. At time of this communication in only 4 cases there was complete recovery. We postulate that the constant bp administration may increased the risk of osteonecrosis in a multifactor etiology. Because this complication rarely cure and is associated with poor quality of life we suggest to investigate the optimal and safe duration of treatment and to consider and prevent this complication in myeloma patients.

PU-031
EVALUATION OF GROWTH FACTORS OBTAINED IN VITRO BY ACTIVATION OF PLATELET RICH PLASMA (PRP)
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Introduction. Thanks to the role of platelet growth factors (PDGF, TGF beta1, EGF, IGF I and II and VEGF) in the processes of tissue repair, platelet gel has been introduced into clinical use. In fact, platelets produce many growth factors (GF) capable of stimulating the reproduction of mesenchymal stem cells, fibroblasts, osteoblasts and endothelial cells, as well as having a chemotactic activity for macrophages, monocytes, mononuclear cells and polymorphonuclear cells. Research has, therefore, concentrated on the possibility of supplying a strong regenerative stimulus to tissue by applying very concentrated platelets in situ. In fact the PRP, after activation, releases numerous GF capable to accelerate the tissue regeneration and used for non conventional treatment of ulcers and surgical wounds. The aim of this study was to evaluate the existence of a correlation between age, sex and platelet count (PLT) of blood donors with the detectable amount of GF in PRP. METHODS PRP was prepared in Caserta’s Hospital and in University, separating one unit of whole blood, from 225 healthy donors (162 men and 63 women), aged 19-59 years. In order to prepare the gel, 10 mL of PRP were mixed with 1 mL of calcium gluconate and 1 mL of human thrombin. This mix was incubated at room temperature for 5 minutes. GF concentrations and PLT count were assayed in whole blood and in PRP before activation. The GF were assayed in the PRP before and after activation. RESULTS The mean PLT count in PRP was 5 times higher than in whole blood (1.54±512 vs. 280±55 x10(3)/mircroliters). Some GF showed high concentrations respect basal values: PDGF-AB = 134±26 ng/mL, TGF-beta1 = 172±69 ng/mL and IGF-I = 96±29 ng/mL; while other GF were only found in little amount: PDGF-BB = 0.32±0.07ng/mL and TGF-beta2 = 0.9±0.5 ng/mL. No influence by donor’s sex or age on GF was discovered (except for IGF-1). GF in PRP showed a slight correlation with PLT in PRP (r <0.05), but not with PLT in whole blood (p=0.35). DISCUSSION GF in PRP showed substantial variations among studied subjects, but the factors influencing their concentrations aren’t still fully known. PLT in PRP showed an initial linear positive relation with GF until to go to a plateau. Nevertheless the standardization of procedures for PRP preparation will allow us to resolve this problem, in fact the immunohematological laboratories will be able to prepare PRP with the desired concentration of platelets in order to obtain the wished clinical results.

PU-032
LENALIDOMIDE IN 5 Q- SYNDROME: A SINGLE CENTRE EXPERIENCE
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Introduction. The 5q- Syndrome is a Myelodysplastic Syndrome (MDS) with severe macrocytic anemia, frequent thrombocytosis, typical dysmegakaryopoiesis and favorable prognosis. Until the advent of lenalidomide, repeated RBC transfusions were the only treatment. Methods. Two patients with 5q- Syndrome, diagnosed in our Institution according to WHO Classification and IPSS Scoring System, were treated with Lenalidomide at 10 milligrams p.o. 21 days/month. Time to transfusion independence was calculated in weeks since the beginning of therapy. Results. FM 66 years-old man, with del 5q31 in 25% of metaphases was diagnosed in October 2004: severe anemia (Hb 4.8 g/dl) and thrombocytosis (PLT 1.200 x10(12)/microliters) were present. Transfusion requirement was 2 units of packed RBC/month. Lenalidomide treatment started in January 2006 but it was interrupted after the first cycle due to a temporary unavailability of the drug. In January 2007 the treatment was resumed. Transfusion independence was reached after 4 weeks. Hemoglobin was > 12 g/dl after 12 weeks. No relevant side effects were noted. In April 2007, cytogenetic analysis showed the absence of del 5q31 in 100% of metaphases. LP 50 years-old woman, with del 5q31 in 80% of metaphases, was diagnosed in December 2006. The patient showed anemia (Hb 8.0 g/dl) and thrombocytosis (PLT 425 x10(12)/µl). Transfusion requirement was 4 units of packed RBC/month. Lenalidomide treatment started in February 2007. A grade 2 neutropenia delayed the beginning of the second cycle by 2 weeks. Transfusion independence was reached after 4 weeks (Hemoglobin 10 g/dl). Cytogenetic evaluation is still ongoing. Discussion. Response to Lenalidomide was rapid. The two patients reached transfusion independence and normalization of platelets count within the first month of treatment. In FM cytogenetic remission was seen after the third month. Despite the interruption of the therapy on January 2006, FM maintained transfusion independence for 32 weeks (average hemoglobin level >12 g/dl). From October 2006 the transfusion requirement was lower compared with pre-Lenalidomide period (1 unit of packed RBC/month). The retreatment produced a new clinical response and a complete cytogenetic remission. These data show the dramatic therapeutic effectiveness of Lenalidomide in this specific type of MDS and suggest the hypothesis of a prolonged 5q31- clone inhibition without any possible event of drug resistance.

PU-033
MAINTENANCE THERAPY WITH GEMTUCUMAB OZOGAMICIN (MYLOTARG) AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Introduction. Acute Myeloid Leukemia (AML) is a disease predominantly affecting older adults, with a median age of 65 years. These
patients represent a poor risk population with a high chemotherapy-related mortality. Standard of care in the management of elderly patients with AML includes combination therapy with cytarabine plus fludara- bine and an anthracycline and can result in complete remission (CR) rate of 40% to 60%. Even if successful, patients relapse quickly. Aggressive postremission therapy does not appear to improve survival. Future direc-tions include therapies targeted at immunomodulation, and, among these newer treatments, Gemtuzumab ozogamicin (GO) has given promising results in relapsed, refractory and untreated CD33+ AML as monotherapy or combination regimens or adjunct to conditioning regi-mens for Stem Cell Transplantation (SCT). Methods. In this study we ana-lyzed the efficacy and the safety of GO as maintenance treatment after Autologous-SCT in three elderly patients (2 males and 1 female; 64, 67 and 69 years, respectively) with CD33+ AML in 1st (2 patients) or in 2nd (1 patient) CR. After a complete hematopoietic engraftment (at least 6 weeks from ASCT), GO was administered alone for 4 doses with 28 days between doses (two patients received 6 mg/mq for the two first doses and 3 mg/mq for the two last doses; the last received 3 mil-ligrams/mq for four doses). Patients were evaluated for Complete Com-plete Remission (CCR) and therapy-related adverse events. Results. All patients are in CCR at +18, +14, +12 months, respectively. Neutropenia (between 500 and 1000 /microliters) and thrombocytopenia (between 50,000 and 100,000/microliters) were observed in two (when GO was given at a dose of 6 mg/mq) and in three patients. No grade 3 or 4 liver toxicity was observed. Discussion. GO administered in fractionated dos-es as maintenance treatment after ASCT in older patients with CD33+ AML in first or second CR demonstrated a good efficacy and an accept-able safety profile.

**PU-035**

**BRONCHIAL ASSOCIATED LYMPHOID TISSUE (BALT) LYMPHOMA: PITFALL IN DIAGNOSIS. A PARTICULAR CASE REPORT**


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Bronchial associated lymphoid tissue (BALT) lymphoma is a distinct subgroup of low grade B cell extranodal non Hodgkin lymphoma (NHL), a rare disease classified as a marginal zone lymphoma (MZL). BALT lympho-ma is usually characterized by an indolent outcome, prolonged time single site localization and late systemic involvement. At onset it still remains difficult to make a correct early diagnosis because of its particu-lar clinicopathological presentation. We report here the clinical course of a BALT LNH accrued in a 30 year old woman. The patient was referred to anot-her institute in August 2002, because of fatigue and haemolytic anemia. A CT scan showed several nodules and interstitial infiltrate of lung with no chest lymphadenopathy or mediastinal involvement. On the first lung infiltrate biopsy a hyalins granuloma diagnosis was made. In March 2003 the patient was hospitalized at our institute because of haemolyt-ic anemia recurrence which was unresponsive to steroid treatment while the chest CT scan remained unmodified. The patient underwent second thoracotomy. During surgery a retromediastinal lung mass not clearly detectable at CT was evidenced and biopsied: on this basis a correct diagnosis of BALT lymphoma was made. From March to September 2003 the patient was given combined immuno-chemotherapy - (R-CHOP) for six cycles achieving marked reduction of lung lesions. Indi-rect Antiglobulin test remained persistently positive without anemia. Thus patient, considered in good partial response, in November 2003 underwent high dose chemotherapy followed by autologous peripher-al blood stem cell transplantation. In February 2004 a disease restaging was done with the aim of performing a consolidation treatment with Radiotherapy. At chest CT scan a minimal residual lung mass, localized between the heart and spine was evidenced. Since involved field radio-therapy would have encompassed these particular sites with possible serious toxicity risk, it was decided to stop any treatment approach to observe the patient. During follow up the chest CT scan were unmodi-fied. As of April 2007 the patient is well, in complete response for 42 months+. It appears that transbronal or transthoracic biopsy and mediastinoscopy are mandatory diagnostic procedures for obtaining a definitive diagnosis in BALT lymphoma. This rare entity tends to be localised in the lung at the time of diagnosis, to respond well to systemic combined chemotherapy, and to have favourable prognosis.

**PU-036**

**IS THALIDOMIDE A RISK FACTOR FOR DEVELOPING A CYTOMEGALOVIRUS INFECTION AND/OR DISEASE IN PATIENTS AFFECTED BY MULTIPLE MYELOMA?**


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Introduction. Despite profound immuno-suppression associated with newer therapies for hematologic malignancies, little information exists regarding cytomegalovirus (CMV) reactivation and disease in settings other than allogeneic stem cell transplantation (SCT). Drugs with anti-angiogenic and proteasome inhibitor activity, such as Thalidomide and Bortezomib, are currently used as therapeutic strategy for Multiple Myeloma (MM). The aim of our study was to evaluate whether the use of Thalidomide represents a risk factor for CMV infection and /or disease in MM unrolled in therapeutic program including autologous SCT (ASCT). Methods. Between April 2006 and April 2007, 14 patients with MM were eligible for ASCT and 18 ASCTs were performed, whom 4 double transplants. At diagnosis, median age was 51 (42-63); 8 received Thalidomide (n=5) + Bortezomib and 6 VAD regimen. Disease status before transplant was CR, PR and MR in 8, 5 and 1 patient, respecti-vely. According to our procedure for MM, pp65Ag and/or plasma CMV- DNA were determined only when CMV was suspected. Results. Over-all, 13 out of 14 patients underwent ASCT, while one of them became not eligible transplant procedure because of severe CMV gut disease after Thalidomide therapy. All transplanted patients engrafted with a median time of PMN recovery of 12 days (11-20) and 2/13 patients devel-oped CMV infection after ASCT. Absence of CMV infection was
observed in the group of patients treated with VAD + ASCT, while CMV infection occurred in 2/7 patients treated with Thalidomide + ASCT. In particular, one patient showed delay of PMN recovery associated with diagnosis of CMV infection on bone marrow specimen (CMV-DNA assay) on day +16; she promptly started pre-emptive therapy and engrafted on day +20. The other patient showed persistent fever of undetermined origin; she achieved PMN engraftment on day +12 and CMV infection was detected by pp65Ag assay on peripheral blood speci- men on day +13. CMV was successfully treated in all 3 cases. 

**Introduction.** ASCT with intermediate or high doses of Melphalan is the most effective therapy in patients with MM being < 65 years old. Several series of non-pluritreated patients are required. In our study we evaluated the effectiveness and toxicity of MVTD conditioning therapy with stem cell support in MM patients (6 males and 6 females: median age 62 yrs - range 46-70) who received ASCT with MVTD conditioning therapy (Melphalan 100 milligrams/m2 days - 6 and - 3, Bortezomib 1.3 milligrams/m2 days - 6 and - 3, Dexamethasone 40 mg/m2 day 0). All patients were in CS III A; one was treated with Rituximab Administration at day 0. In the first group of patients an additional chemotherapy was administered with a median of 3 days (1-5); all had received previously a therapy with Thalidomide, two Thalidomide + Velcade, one PEG-IFN. Results. 100% of patients obtained a response: 2 patient (16.7%) obtained CR, 4 (33.3%) a nCR, 3 (27.8%) a VGPR and 1 (16.7%) a PR. One patient in CR was subjected to 2 therapeutic lines, 4 in nCR from 2 to 4, 4 in VGPR from 2 to 4. At the moment, after a median of 7 months (1-19), 4.53% (2/44) still keep the obtained response with a medi- an of 7 months (1-19), 2 (27.8%) after progression are in rescue therapy, 2 pts (16.7%) died because of progression. Progression Free Survival median since MVTD beginning is of 5 months (1-24). None of the patients have developed a significant neurotoxicity. All patients have developed grade 4 thrombocytopenia and neutropenia, with a median of 3 days (1-10) and 6 days (4-12), respectively. 28.3% of patients had neutropenia with fever with a median of 2 days (0-2). The median units of platelets and red cells infused was 2 (2-11) and 2 (0-10), respectively. Conclusions. In our experience, ASCT with MVTD conditioning, appeared to be effective in relapsed patients with MM. Although in a small series of patients the response obtained was independent from the number and type of previous therapies and from the clinical stage, none neurotoxicity has been observed, whether the haematological toxicity was overlapping to that due to conventional ASCT. Further studies, in larger series of non-pluritreated patients are required.

**PU-037**

**AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AFTER CONDITIONING THERAPY WITH INTERMEDIATE DOSE MELPHALAN, BORTEZOMIB, THALIDOMIDE, DEXAMETHASONE (MVTD) IN RELAPSED MULTIPLE MYELOMA (MM) PATIENTS**


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**Introduction.** ASCT with intermediate or high doses of Melphalan is the most effective therapy in patients with MM being < 65 years old. Several studies have shown that the synergic effect of Bortezomib and Thalidomide in association with conventional chemotherapies, induces a more pronounced reduction of tumour mass and an enhancement of clinical responses. In our study we evaluated the effectiveness and toxicity of MVTD conditioning therapy with stem cell support in MM relapsed patients. Results. From January 2005 to February 2007, 12 patients (6 males and 6 females: median age 62 yrs - range 46-70) received ASCT with MVTD conditioning therapy (Melphalan 100 milligrams/m2 days - 6 and - 3, Bortezomib 1.3 milligrams/m2 days - 6 e - 3, Thalidomide 200 milligrams from day -6 to day -2, Dexamethasone from day - 6 to - 3, CSP infusion at day 0). All patients were in CS III A; median performed therapies 3 (1-5); all had received previously a therapy with Thalidomide, two Thalidomide + Velcade, one PEG-IFN. Results. 100% of patients obtained a response: 2 patient (16.7%) obtained CR, 4 (33.3%) a nCR, 3 (27.8%) a VGPR and 2 (16.7%) a PR. One patient in CR was subjected to 2 therapeutic lines, 4 in nCR from 2 to 4, 4 in VGPR from 2 to 4. At the moment, after a median of 7 months (1-19), 4.53% (2/44) still keep the obtained response with a medi- an of 7 months (1-19), 2 (27.8%) after progression are in rescue therapy, 2 pts (16.7%) died because of progression. Progression Free Survival median since MVTD beginning is of 5 months (1-24). None of the patients have developed a significant neurotoxicity. All patients have developed grade 4 thrombocytopenia and neutropenia, with a median of 3 days (1-10) and 6 days (4-12), respectively. 28.3% of patients had neutropenia with fever with a median of 2 days (0-2). The median units of platelets and red cells infused was 2 (2-11) and 2 (0-10), respectively. Conclusions. In our experience, ASCT with MVTD conditioning, appeared to be effective in relapsed patients with MM. Although in a small series of patients the response obtained was independent from the number and type of previous therapies and from the clinical stage, none neurotoxicity has been observed, whether the haematological toxicity was overlapping to that due to conventional ASCT. Further studies, in larger series of non-pluritreated patients are required.

**PU-039**

**THE RISK OF SEPSIS, LINKED TO PLATELET TRANSFUSIONS, IS GREATER THAN THE COMBINED RISK OF TRANSMITTING HIV, HBV OR HCV**

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**Introduction.** About 10% of transfusion-associated deaths are caused by sepsis due to bacterial contamination of hemocomponents, particularly of platelet concentrates (PC). In fact PC are stored in conditions allowing bacterial growth: at 22°C in nylon of plasma and in particular bags allowing oxygen exchange. The main sources of contamination are the skin flora, introduced into the blood unit during donation, and an occult donor bacteraemia. About 70-80% of isolated bacteria are Gram+, mainly Staphylococcus and Streptococcus, while Gram- comprise prevalently Serratia, Salmonella and Yersinia. Aim of this study has been to evaluate the risks of bacterial contaminations of PC in our Country, in order to determine if new strategies for blood donation and transfusion are necessary. METHODS: Transfusion of a contaminated PC generally does not produce clinical consequences, nevertheless, in some cases, it can cause bacteremia, fever, chills, hypotension, nausea, vomiting, diarrhea and oliguria, which may progress to sepsis and ultimately multi-system organ failure and death. Our statistical evaluation has been based on the basis of Italian Health Superior Institute’s data and on international reports. RESULTS: It has been calculated that 1.200-1.800 blood units could have been contaminated in one year, about 6.931-10.397 contaminated blood units could have been collected in our Country and 244-1.040 transfusion related sepsis, with 41 fatalities, could have been taken place. As a similar residual risk of sepsis and fatality is largely greater than the combined risk of transfusing HIV, HBV and HCV. Discussion. Considering the clinical significance and the fatal risk of bacterial contamination, two precautionary measures must be taken: 1) to prevent a bacterial contamination; 2) to avoid the not necessary transfusions, especially of random PC. The first point
forces all Transfusion Centres to routinely apply a stringent donor screening and a careful disinfection at the phlebotomy site, and to use a sterile connector for laboratory manipulations. The second point mainly involves haematologists, paediatrics and oncologists that largely prescribe platelet transfusions.

**PU-040**

**BORTEZOMIB AND RITUXIMAB (R-VELCADE) IN RELAPSED AND REFRACTORY NON HODGKIN’S LYMPHOMA**

Merla E, La Sala A, Dell’Olio M, Bodenizza A, Carella AM, Falcone A, Greco MM, Mantuanu S, Melillo L, Nobile M, Sanpaolo G, Scalzulli PR, Cascavilla N

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**Introduction.** Bortezomib, a powerful and reversible inhibitor of proteasome 26S, exhibited a defined therapeutic activity in Multiple Myeloma. Significant responses have been recently reported also in some non Hodgkin’s Lymphomas (NHL) like Mantle cell lymphoma (MCL) and advanced or refractory Follicular Lymphoma (FL). Methods. Four patients with FL and 6 with MCL, have been treated in our clinical center with Bortezomib aiming to verify its efficacy in the treatment of advanced or refractory NHL. All patients were in an advanced phase (Clinical Stage III or IV) and had already received multiple treatment: i.e., 2 to 5 chemotherapeutic lines; in 4 cases the relapse appeared after Autologous Stem Cell Transplantation (ASCT). All patients were treated with Rituximab 375 milligrams/mq at day 1 and Bortezomib 1.3 milligrams/mq at day 1, 4, 8 and 11. The therapeutic design was repeated every 21 days. Results. FL patients: Out of the 4 patients that have been treated (mean age: 57 years range 54-59 years), two achieved a Complete Remission (CR) and one a good partial remission; while no response was observed in the last patient. Hematological toxicity (thrombocytopenia grade 2) was shown mostly after the third cycle. In one single patient a grade 3 neurotoxicity was observed (at present in resolution). MCL patients: Out of the 6 patients that have been treated (mean age: 71 years range 61-79 years), 3 achieved a CR, one was refractory and 2 (the eldest) had to suspend the treatment after the second cycle cause of a severe pan-cytopenia. The overall non-hematological toxicity was negligible. None of the patients had significant gastro enteric toxicity; in a single patient a grade 3 neurotoxicity was observed after the fourth cycle of therapy: this latter patient had to delay the fifth cycle of about 3 weeks and reduce the Bortezomib dose (0.7 milligrams/mq). A patient, after CR, was subjected to ASCT and is still in CR after 3 years. Discussion. In advanced stage or refractory to conventional chemotherapy NHL patients, either FL and MCL, Bortezomib was shown to be therapeutically greatly effective. We favour an earlier use of the above methods.

**PU-042**

**NON-HAEMOLYTIC FEBRILE TRANSFUSION REACTION AFTER PLASMA REDUCTION OR LEUKODEPLETION OF PLATELET CONCENTRATES IN NEWBORNS AND CHILDREN**

Misso S,1 Paesano L,1 D’Onofrio M,1 Fratellanza G,2 D’Agostino E,3 Feola B,1 Fratellanza A,1 Marotta S,1 Minerva A1

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**Introduction.** The non-haemolytic febrile transfusion reaction (NHFTR) complicates 2 to 37% of transfusions of random platelet concentrates (RPC) in adults. However RPC are frequently transfused in newborns and children affected with a severe thrombocytopenia too, but the incidence of FNHTRs is not well known. In this study, carried out in Caserta’s Hospital, we have just evaluated the incidence of FNHTRs after platelet transfusion in newborns and in children. In addition, in order to identify a technical procedure able in prevent this reaction, we have also compared the results obtained from 3 different hemocomponents: 1) the standard RPC, obtained from a single donor platelet-rich- plasma; 2) a platelet hyper concentrate (PHC), characterized by reduction of plasma volume after storage; 3) a filtered platelet concentrate (FPC) with leukodepletion before storage. Methods. Children from birth until 12 months of age were eligible for this study, independently from their pathology. For the first aim of this study, we have retrospectively analyzed the platelet transfusions, using unmodified RPC, performed in a period of 6 months. For the second aim, for 3 months we have transfused PHC with reduction of the plasma volume just before transfusion; while for 3 months we have used RPC. In a second step, for 2 months we have used FPC, with a maximum storage of 2 days. Signs and symptoms characteristic of a NHFTR during, immediately after and 2 hours following transfusion were registered. Results. In the considering period, 115 platelet transfusions were administered to 77 children; in particular 57 RPC were transfused to 35 children, 29 PHC to 27 children. The overall non-hematological toxicity was negligible. None of the patients had significant gastro enteric toxicity; in a single patient a grade 3 neurotoxicity was observed after the fourth cycle of therapy: this latter patient had to delay the fifth cycle of about 3 weeks and reduce the Bortezomib dose (0.7 milligrams/mq). A patient, after CR, was subjected to ASCT and is still in CR after 3 years. Discussion. In advanced stage or refractory to conventional chemotherapy NHL patients, either FL and MCL, Bortezomib was shown to be therapeutically greatly effective, with a CR proportion of 50%. Overall, it was well tolerated and devoid of important toxicity. Only in elderly MCL patients, with a more intense previous treatment and a worse performance status, the hematological toxicity was more intense. We favour an earlier use of the above design, possibly in wider multicentric studies.

**PU-041**

**PEGFILGRASTIM TO SUPPORT CHOP-R CHEMOTHERAPY ADMINISTERED EVERY 14 DAYS IN THE PATIENTS (PTS) WITH AGGRESSIVE B-CELL NON-HODGKIN’S LYMPHOMA (NHL)**

Capocchiani E,1 Cupini S,1 Barbara C,1 Fontana A,1 Loupakis F,1 Safina V,1 Vasile E,1 Bursi S,1 Colteli L,1 Landi L,1 Baldi GG,1 Barletta MT,1 Giuntini N,1 Lo Dico M,1 Mazzoni E,1 Misso S,1 Paesano L,1 D’Onofrio M,1 Fratellanza G,2 D’Agostino E,3 Feola B,1 Fratellanza A,1 Marotta S,1 Minerva A1

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**Background.** CHOP chemotherapy administered every 21 days has been the standard regime for treatment of aggressive NHL for many years. Recent studies have shown improvements in both complete remission and survival following addition of Rituximab to CHOP 21 (Coffier et al, NEJM 2002) and following reduction of the cycle length of standard 21 day CHOP to 14 days (Frenschushc et al, Blood 2004). Previous studies with CHOP 14 have demonstrated the need of filgrastim to allow the administration of chemotherapy at planned dose and on time in this setting. Methods. This study evaluates the feasibility of the combination of CHOP plus Rituximab (375 mg/mq) repeated every 2 weeks (CHOP-R 14) supported with a single administration pegfilgrastim (6 mg, on day 2) up to six cycles in pts with aggressive B-cell NHL. Results. Up today 18 pts have been enrolled. Patients’ characteristics are: median age 66.5 years (46-75), sex 8M/10F, ECOG PS 0(14), 1(3), 2(1), B symptoms 39%, stage I(1), II(6), III(6), IV(5), extranodal location 4 (22%). Overall 94% of pts received full dose chemotherapy on schedule for all planned cycles (range 4-7). Chemotherapy was delayed and dose reduced in 11% and 6% of pts respectively. Main grade 3-4 adverse events per pts were: neutropenia 35%, febrile neutropenia 11%, thrombocytopenia 11%, anemia 22%. One pts died because of treatment related sepsis. Responses at the end of treatment (14 evaluable pts, 4 too early) were: complete 71%, partial 3%. Up today the median follow up is 7.1 months, no pts progressed and median RFS and OS have not been reached. Conclusions. These preliminary results indicate that the delivery on schedule of dose-dense CHOP-R 14 to pts with previously untreated aggressive B-cell NHL is safe and active with once per cycle pegfilgrastim support.

**PU-043**

**ONSET OF SECOND PRIMARY CANCER AFTER HODGKIN’S DISEASE: REVIEW OF LITERATURE**

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At present time, Hodgkin’s Disease is one of the most curable cancers. New advances in Radiotherapy and in Chemotherapy help improve the overall survival and cure rates of patients with Hodgkin’s Disease. However, late complications of long-term survivors have a remarkable clinical importance: they include cardiovascular, pulmonary and thyroid dys...
functions, sterility, immunity alterations and the arising of second tumor. Second primary cancer, particularly Acute Non-Lymphoid Leukemia, Non-Hodgkin lymphoma and secondary solid tumors have been used as a known late complications that can occur in patients treated for Hodgkin’s Disease. The risk of Second primary cancer after radiation therapy is well known from studies of cancer patients treated by Radiotherapy and from investigations of other groups exposed to radiation. The risk of cancer after Chemotherapy is less clear, however, because the use of Chemotherapy is more recent and many different therapeutic agents have been used. In comparison with the tumor tissue, the risk of Second primary cancer is highly increased in the patients treated at younger age for Hodgkin’s Disease. The increase of Acute Non-Lymphoid Leukemia risk is higher in patients treated with combined Radiotherapy and Chemotherapy regimens including mechloretamine and procarbazine and the risk reaching a peak in the early years after exposure, and that of various solid tumors increases later. Non-Hodgkin Lymphoma’s arising is set in relation with the radiochemotherapy treatment: the risk has concentrated in the first year following start of treatment and declined in the subsequent 5 years. The absence of relation between received treatment, age and appearance of Non-Hodgkin Lymphoma is also mentioned in literature. Regarding to the occurrence of Acute Non-Lymphoid Leukemia after Radiotherapy alone and the potential role of sex, age, splenectomy and spleen irradiation, further comparison with prolonged follow-up are needed in patients with similar treatment. Moreover, further studies are needed to determine the potential role of the spleen in tumoral immunosurveillance: for example, the clinical, histologic and immunophenotypic features in patients with Non-Hodgkin’s Lymphoma were similar to those of individuals with immune deficiency. If the initial triggering of the cell-mediated immune response takes place in the spleen and the lymph nodes, it could be thought that the immunosuppression occurring at splenic tissue is an important, though not complete, damage of tumoral immunosurveillance capabilities. With regard the onset of secondary Non-Hodgkin’s Lymphoma, the immunodeficiency is peculiar for Hodgkin’s Disease and spleen irradiation inducing functional hyposplenia, and it is conceivable that the immune defect of chronic T-cell may permit a clonal proliferation of B-cell, and may increase the immunosuppression of Hodgkin’s Disease itself: in fact, the Non-Hodgkin’s Lymphoma is one of the most frequent tumors observed in individuals with immune suppression. Moreover, further studies are needed to determine the potential role of the spleen in tumoral immunosurveillance:

### PU-044

**BORTEZOMIB IN RESISTENT EXTRAMEDULLARY PLASMACYTOMA TREATMENT: A CASE REPORT**

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Extramedullary plasmacytomases (EMP)s are uncommon variant of plasma cell dyscrasia, with a worldwide annual incidence of 3 per 100,000 population. They account for 1% of all tumors of the head and neck and 4% of all nonneoplastic tumors of the nasal tract. Approximately 80-90% of them involve the the Mucosa-Associated Lymphoid Tissue of the upper airways, 75% of these involve the nasal and paranasal regions. Males are more frequently affected during the fifth and sixth decades. Radiotherapy is the treatment of choice, surgery being limited to biopsy and to excision of residual disease. Bortezomib is a first class potent and reversible proteasome inhibitor approved for the treatment of relapsed and/or refractory multiple myeloma. We report the case of 70 year-old female patient with the nasal cavity plasmacytoma. At diagnosis (2005, November), the patient showed a mass lesion in the left nostril. No cervical lymphonode swelling is found. MRI revealed a 2.5×1.5 cm mass with the septum erosion with pathological tissue extension. The lesion was limited to biopsy and to excision of residual disease. Bortezomib was administered at dose of 0.7 mg/m2 in the same days in 7th and 8th course because peripheral neuropathy (pain and paresthesias grade 3). No other toxicity was observed. MRI control showed a complete resolution of the initial lesion. Our single observation suggest that the combination of Bortezomib, Melphalan and Dexamethasone seems have good activity in extramedullary plasmacytomase resistant to the radiotherapy.

### PU-045

**PEGYLATED FILGRASTIM VERSUS UNCONJUGATED G-CSF PLUS CHEMOTHERAPY FOR THE MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS: RESULTS OF A RANDOMIZED TRIAL IN LYMPHOMA AND MYELOMA PATIENTS**


Department of Haematology; 1Department of Transfusion Medicine; 2Laboratory of Clinical Pathology, Hospital Card. G. Panico, Tricase (Le), Italy

High-dose chemotherapy followed by autologous blood stem cell transplantation are widely used in myeloma and lymphoma. Recently, pegfilgrastim seems as effective as G-CSF in mobilizing CD34+ stem cells after chemotherapy and optimizing the engraftment potential of pegfilgrastim mobilized CD34+ cells. In this randomized study (1:2) we evaluate the efficacy of pegfilgrastim plus chemotherapy compared to non pegylated G-CSF in mobilizing peripheral blood CD34+ cells in patients with lymphoproliferative disorders. **Methods.** From March 2007 to April 2007, 47 patients (52 non Hodgkin Lymphoma - NHL, 5 Hodgkin Disease - HD, 10 Multiple Myeloma - MM) were randomized to receive a single dose of 6 mg pegfilgrastim on day 4 (median, range 2-7) or daily doses of G-CSF (10 micrograms/Kilogram/day) from day 4 (median, range 2-5) after mobilization chemotherapy for a total of 56 harvesting procedures. **Results.** Table 1 shows the clinical characteristics of patients.
of patients enrolled (20 with peg-filgrastim and 36 with G-CSF). No significant differences regard clinical characteristics are tested between the two groups. A median of 2 apheresis (range 1-3) was performed, see Table 2. Higher CD34+ count in the peripheral blood was 5×10^6 (median, range 1.06-24.88) in the pegfilgrastim and 6.9×10^6 (1.09-32.8) in the G-CSF group, (p=0.25). The total number of collected CD34+ cell/kilogram was 3×10^6/kg (median, range 1.06-32.84) in the pegfilgrastim and 6.9×10^6 (1.06-24.88) in the G-CSF group, (<0.01). Mobilization failure occurred in 18% of cases (1/20 in peg-filgrastim and 9/36 in G-CSF, p=0.05) and 23% of cases reached <2 CD34×10^6 (10% and 31% in the peg-filgrastim and G-CSF, p=0.05). Twenty-seven patients (55%) were autotransplanted (12 and 15 patients in peg-filgrastim and G-CSF arm, respectively). Patients mobilized by peg-filgrastim infused a median of 5.56×10^6 CD34+ cell/Kg (range 1.04-20.25) in comparison to 6.04×10^6 CD34+ cells/Kg (1.9-21.6) in patients mobilized by G-CSF (p<0.01). Despite the lower number of CD34+ cells, there was no significant difference regarding to the time need for haematological reconstitution, see Table 3. Not significant differences were tested in the immunological reconstitution at day +90, Table 3. Twenty-one patients (87%) experienced a febrile neutropenia (range 1.04-20.25) in comparison to 6.04×10^6 CD34+ cells/Kg (1.9-21.6) in patients mobilized by G-CSF. The early death rate before day 90 was 27% on the PEG arm and 33% (43% in the peg-filgrastim and 57% in the G-CSF group, not significant differences were tested in the immunological reconstitution at day +90, Table 3. Not significant differences were tested in the immunological reconstitution at day +90.

### Table 1. Characteristics of patients enrolled.

<table>
<thead>
<tr>
<th></th>
<th>AI</th>
<th>Pegylated-GCSF</th>
<th>G-CSF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of procedures</td>
<td>56</td>
<td>20 (36%)</td>
<td>36 (64%)</td>
<td></td>
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<tr>
<td>Age, median (range) years</td>
<td>52 (15-75)</td>
<td>52 (16-68)</td>
<td>50.5 (15-76)</td>
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<tr>
<td>Gender, n (%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>28 (50%)</td>
<td>9 (32%)</td>
<td>19 (68%)</td>
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<tr>
<td>Female</td>
<td>28 (50%)</td>
<td>11 (39%)</td>
<td>17 (61%)</td>
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<td>Diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non Hodgkin Lymphoma</td>
<td>38 (68%)</td>
<td>12 (30%)</td>
<td>26 (70%)</td>
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<tr>
<td>Aggressive</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hodgkin Disease</td>
<td>6 (11%)</td>
<td>3</td>
<td>3</td>
<td></td>
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<tr>
<td>Multiple Myeloma</td>
<td>12 (21%)</td>
<td>6</td>
<td>6</td>
<td></td>
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<td>BOM involvement at diagnosis, n (%)</td>
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<td>Yes</td>
<td>19 (42%)</td>
<td>7 (37%)</td>
<td>12 (63%)</td>
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<tr>
<td>No</td>
<td>26 (58%)</td>
<td>8 (31%)</td>
<td>18 (69%)</td>
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<tr>
<td>IPI &gt; 2, n (%)</td>
<td>18 (62%)</td>
<td>4</td>
<td>14</td>
<td>1.15</td>
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<td>FLI &gt; 3, n (%)</td>
<td>4 (44%)</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Number of previous chemotherapy regimens at mobilization, median (range) &gt; 2</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
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<td>Months from diagnosis at mobilization, median (range)</td>
<td>7 (3-192)</td>
<td>11 (3-53)</td>
<td>6 (3-192)</td>
<td>0.9</td>
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<tr>
<td>Mobilization regimen, n (%)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>High Dose Ara-C</td>
<td>38 (68%)</td>
<td>13 (34)</td>
<td>25 (66)</td>
<td>0.35</td>
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<tr>
<td>Cytosinephosphate</td>
<td>12 (21)</td>
<td>6</td>
<td>6</td>
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<td>Other</td>
<td>6 (11)</td>
<td>1 (17)</td>
<td>5 (83)</td>
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<td>Status disease</td>
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<tr>
<td>Responsive</td>
<td>46 (83%)</td>
<td>16 (35%)</td>
<td>30 (65%)</td>
<td>0.5</td>
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<tr>
<td>Not responsive</td>
<td>10 (18%)</td>
<td>4 (14)</td>
<td>6 (60)</td>
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### Table 2. Leukapheresis data.

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<tbody>
<tr>
<td>Median apheresis procedures</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>2 (1-3)</td>
<td>0.36</td>
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<tr>
<td>&lt; 2</td>
<td>41 (68)</td>
<td>15 (26)</td>
<td>26 (64)</td>
<td></td>
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<tr>
<td>&gt; 2</td>
<td>7 (14)</td>
<td>4 (67)</td>
<td>3 (43)</td>
<td></td>
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<tr>
<td>Median day in apheresis</td>
<td>13 (10-21)</td>
<td>13 (10-16)</td>
<td>13 (10-21)</td>
<td>0.67</td>
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<tr>
<td>Maximal peripheral blood cell count per microlitre</td>
<td>28.6 (6.1-492.5)</td>
<td>23.9 (7.21-492.5)</td>
<td>41.16 (1.09-32.8)</td>
<td>0.2</td>
</tr>
<tr>
<td>WBC count at 1st apheresis, median (range)</td>
<td>4790 (1000-34000)</td>
<td>4790 (1000-19700)</td>
<td>4325 (1000-34000)</td>
<td>0.9</td>
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<tr>
<td>Poor mobilize (&lt;202CD34+×10^6/kg)</td>
<td>13 (23%)</td>
<td>2 (10%)</td>
<td>11 (31%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Median CD34+×10^6/Kg</td>
<td>6.5 (1.06-32.84)</td>
<td>5 (1.06-24.88)</td>
<td>6.3 (1.09-32.8)</td>
<td>0.1</td>
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<tr>
<td>Mobilization failure</td>
<td>10 (17.9%)</td>
<td>1 (10)</td>
<td>9 (90)</td>
<td>&lt;0.05</td>
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</table>

### Table 3. Haemopoietic recovery and recovery of CD3+, CD4+, CD8+ and CD56+ cells.

<table>
<thead>
<tr>
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<th>G-CSF</th>
<th>p</th>
</tr>
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<tr>
<td>Neutrophil reconstitution</td>
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<tr>
<td>&gt;0.5x10^9/Litre</td>
<td>11 (7-47)</td>
<td>12 (8-21)</td>
<td>11 (7-47)</td>
<td>n.s.</td>
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<tr>
<td>Platelets reconstitution &gt; 20x10^9/Litre</td>
<td>15 (8.55)</td>
<td>18 (11-43)</td>
<td>14 (8.55)</td>
<td>n.s.</td>
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</table>

### PU-046

**CLINICAL IMPROVEMENT OF GUILLAIN-BARRE SYNDROME AFTER ALLOGENIC STEM CELLS TRANSPLANTATION IN PATIENT WITH CHRONIC MYELOID LEUKAEMIA**


Guillain-Barre syndrome (GBS) is a group of autoimmune syndromes consisting of demyelinating and acute axonal degenerating forms of the disease. We described a case of a patient with chronic myeloid leukaemia and GBS, improved after allogeneic stem cells transplantation. The patient (male, 46 years old) had the diagnosis of chronic myeloid leukaemia at the age of 43 years; treated with STI 587, after 3 years his condition improved in accelerated disease stage. He underwent chemotherapy with VP-16 and Ara-C and obtained a long phase of aplasia with under-capsular splenic haematomatia with following splenectomy. The clinic assessment was complicated by a mixed motor sensitive polyradiculitis. Patient exhibit a progressive paralysis that reaches a plateau phase. NMR and EMG studies confirmed GBS. The patient underwent HLA sibling donor transplantation in May 2006. In order to reduce the drug toxicity, the conditioning was with Thiopeta, Fludarabine and Melphalan, followed by allogeneic depleted T and B PBSC infusion, without CoVHD prophylaxis. Engraftment was at day +14 (Neutrophils >0.5x10^9/L). The post-transplant program included donor lymphocyte infusion in order to speed up the immunological reconstitution for the infectious risks and disease’s control. Also, he was treated with DLI every 3-4 weeks (total: 5 infusions). After the follow-up at 330 days, the patient shows a quantitative chimerism of 83% with rearrangement BCR/ABL of 5000 copies. From a clinic and neurological point of view, the patient obtained a gradual neurological recovery, documented by neurological revaluation. At the moment, has been documented the recovery of the deambulation.
autonomy, with a support, and a fair recovery of the upper limbs muscular strength. We observed a clinical improvement associated with the recovery of CD45RO-positive CD4+ or CD8+ T-cell subsets.

**PU-047**

**VELCADE AS RE-TREATMENT OF RELAPSING MULTIPLE MYELOMA PATIENTS**

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**Introduction.** In the randomized phase 3 trial (APEX) including 669 patients with relapsed multiple myeloma (MM) patients velcade has proven more effective than high-dose dexamethasone, as demonstrated by a significant improvement in response rate (43% vs 18%), median time to progression (6.2 vs 5.4 months) and 1-year survival rate (80% vs 67%), respectively. Its use in addition to dexamethasone in patients witnessing a suboptimal response to velcade alone has been associated with an improvement in responses without prohibitive toxicity, suggesting that the combination of these two drugs can overcome drug resistance. **Methods.** To evaluate the possibility of re-inducing a response in relapsed patients previously treated with velcade, we re-treated 3 patients with the same drug at 1.3 mg/m² administered on days 1, 4, 8 and 11 of a 21-day treatment cycle for 8 cycles and dexamethasone 20 mg on days 1-2, 4-5, 8-9 and 11-12. **Results.** All patients — who had been previously treated with velcade as single agent — obtained after 47, 54 and 93 months, respectively, from diagnosis and after more than two lines of previous therapy a very good response without a complete disappearance of the monoclonal component (MC). They were thereafter observed and re-treated with the same drug plus the dexamethasone for a relapse documented after 7, 13 and 23 months, respectively. Velcade was well tolerated and the toxicity acceptable, without additional adverse events compared to the first administration. No patient experienced a herpes zoster reactivation and all side effects were typically reversible and disappeared upon drug suspension. Response was rapid with a decrease of the MC of 96%, 86% and 57% after 7, 6 and 5 cycles, respectively, and with absence of cumulative toxicities. At the moment, 1 patient has experienced an increase of the MC after 7 months, 1 is still responsive after 8 months and the last showed disease progression and died 5 months later. **Discussion.** The responses observed in our relapsed MM patients who received velcade for the second time are encouraging; however, in view of the relapses observed, a maintenance program should be considered. It can be hypothesized that velcade, which offers great promise to overcome resistance to conventional chemotherapy, may not be susceptible to the most common drug resistance mechanisms. A longer follow-up and a greater number of patients will conclusively confirm the true efficacy of velcade as re-treatment drug.

**PU-048**

**MULTIPLE SITES RELAPSES OF EXTRANODAL NON HODGKIN LYMPHOMA IN A PATIENT WITH HCV INFECTION: CLINICAL COURSE, HYSTOLOGICAL TRANSFORMATION AND DEVELOPING OF SECONDARY POLYCYTIA VERA.**


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The incidence of extranodal non-Hodgkin Lymphoma (NHL) is increased in patients with chronic hepatitis C virus (HCV) infection. We report a patient with extranodal NHL presenting multiple relapses with primary myelofibrosis. A 55-year-old male presented with a big mass in left thigh and right arm. Biopsy proved again a large B-cell NHL relapse. She started DHAP regimen with a good response after three cycles. In addition in 2006 she developed a JAK2 positive Polycytia Vera. Our observations confirm that HCV can be a trigger for clonal B cells proliferation also related to MALT NHL, they show that MALT NHL can progress to more aggressive disease such as large B-cell subtype, and that polycytia vera is a rare but possible secondary neoplasm due to expansion and dysregulation of myeloid progenitors as a consequence of drug-induced acquired somatic mutations.

**PU-049**

**TREATMENT OF MYELOMATOUS PLEURAL EFFUSION WITH INTRAPLEURAL BORTEZOMIB. A CASE REPORT**

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Myelomatous pleural effusion (MPE) is a rare and severe complication of the clinical course of multiple myeloma (MM). There is no standard treatment for MPE and despite aggressive local and systemic treatment the survival is generally less than four months. Bortezomib has shown an impressive activity in patients with relapsed/refractory MM. We describe a case of MPE successfully treated with intrapleural plus intravenous bortezomib administration. A 66-year-old, IgGκ MM male patient, while in progression after tandem autologous bone marrow transplantation, was admitted to our hospital because of severe respiratory distress. A thorax CT scan revealed a left pleural effusion and a 5 cm nodular lesion involving the upper lobe of the right lung. Morphological, flow-cytometric, and immunocytochemical analysis of the pleural fluid and the echo-guided lung biopsy showed the presence of atypical CD38+ IgGκ plasma cells, and an M-component which was identical to that found in the serum. The patient received two courses of bortezomib (1.3 mg/m²) plus dexametasone (20 mg), intravenously, in a 3-week cycle on days 1, 4, 8, and 11; pegylated liposomal doxorubicin (Caelyx®) at a dose of 40 mg/m² was also administered on day 1 of each cycle. No additional adverse events were registered after intrapleural bortezomib infusion. Pleural fluid progressively cleared and after the second intrapleural dose of bortezomib the pleural fluid turned completely cell-free. A CT scan documented a nearly complete recovery of the pleural effusion. The patient gave his informed written consent to the intrapleural administration of Bortezomib. Thus, on days 1, 4, 8, and 11 of the next two cycles, after performing an evacuative thoracentesis, we infused bortezomib concentration using combined intravenous and intrapleural infusion. The patient showed large B-cell NHL. The patient was treated with CVP regimen (8 cycles) plus 4 cycles of Rituximab, with complete remission. In March 2001 she developed cutaneous-subcutaneous lesion at left lower limb. Histology showed large B cell NHL with JAK2 positive. A CVP regimen (8 cycles) plus 4 cycles of Rituximab, with complete remission. In September 2002 she developed conjunctival relapse in the same eye (histology confirmed large B-cell NHL). She was treated successfully with CEOP regimen (4 cycles). In August 2003 she developed cutaneous-subcutaneous relapse in left arm, treated with CVP regimen (4 cycles) with complete remission. In May 2005 she developed a rapidly increasing cutaneous subcutaneous mass, with capillary angiogenesis, involving nervous roots. Histology indicated relapse of large B cell NHL. The patient was treated with RMiCEOP regimen (4 cycles) with complete clinical and radiological remission. In January 2007 she developed a big mass in left thigh and right arm. Biopsy proved again a large B cell NHL relapse. She started DHAP regimen with a good response after three cycles. In addition in 2006 she developed a JAK2 positive Polycytia Vera. Our observations confirm that HCV can be a trigger for clonal B cells proliferation also related to MALT NHL, they show that MALT NHL can progress to more aggressive disease such as large B cell subtype, and that polycytia vera is a rare but possible secondary neoplasm due to expansion and dysregulation of myeloid progenitors as a consequence of drug-induced acquired somatic mutations.
PU-050
FEASIBILITY AND EFFICACY OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPANTATION IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) OR POOR PROGNOSIS MYELODYSPLASTIC SYNDROME (MDS): A SINGLE CENTER EXPERIENCE

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Introduction. median age of AML and MDS patients (pts) at diagnosis is advanced (65 years). Many elderly pts receive only palliative care as considered too frail to tolerate an aggressive therapeutic approach and their survival is limited to few weeks or months. Conventional chemotherapy provides prolonged survival (>2 years) in only about 10% of pts older than 60. Allogeneic (allo) stem cell transplantation (SCT) is the only potentially curative strategy for AML and poor prognosis MDS, but this procedure is restricted on the basis of patient age because of a high risk of transplant-related mortality (TRM). At our Institute we usually candidate AML/MDS older pts with unfavourable prognosis to alloSCT on the basis of their performance status (PS) and vital organs function. We here retrospectively evaluate the feasibility and outcome of alloSCT in AML and MDS elderly pts transplanted at our Institute. Methods. period 10/2002 to 2/2007, 16 pts, median age 65 (61-72). Diagnosis (WHO): 6 AML, 5 AMLMD, 2 t-AML, 1 RAEB1, 1 RAEB2, 1 MDS/MPD. Cytogenetics: normal 7, intermediate 4, complex 3, not evaluable 2. Status at transplant: complete remission (CR) 6 pts, partial remission 1, refractory to induction 5, relapsed 4. Median number of chemo cycles before transplant: 2 (range 1-7). Previous autologous SCT: 3. Results. 4 patients received their alloSCT from a sibling donor, 10 from an haploidentical family donor or from an unmatch donor. Conditioning: 14 tr eosulfan+fludarabine ±ATG, 1 donor, 10 from an haploidentical family donor.

Discussion: prolonged OS is achievable with alloSCT in elderly pts with diagnosis of AML or poor risk MDS. AlloSCT should be offered to elderly pts selected according to PS and new available comorbidities score systems (e.g. Sorrow index). New treosulfan based conditioning regimen should allow successful transplant in these pts also from alternative donors. Adequate prevention and treatment of infections and effective GvHD prophylaxis could reduce the TRM and improve pts survival.

PU-051
ACETAMINOPHEN-INDUCED HEPATIC LESIONS MIMICKING MASSIVE INVOLVEMENT BY T-CELL/HISTIOCYTE-RICH DIFFUSE LARGE B CELL NON HODGKIN LYMPHOMA (NHL)

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A forty-year old man referred to our Centre due to a fever lasting about one month with continuous assumption of high dose of Acetaminophen. Physical examination did not detect any lymphoadenopaty. Total body CT scan showed enlarged spleen (15 cm) with multiple nodular areas, multiple para-aortic, peripancreatic and hepato-duodenal lymphoadenopaties with no evidence of liver involvement. Laboratory data were in the normal range except for clotting time prolongation. Diagnosis (WHO): 6 AML, 5 AMLMD, 2 t-AML, 1 RAEB1, 1 RAEB2, 1 MDS/MPD. Cytogenetics: normal 7, intermediate 4, complex 3, not evaluable 2. Status at transplant: complete remission (CR) 6 pts, partial remission 1, refractory to induction 5, relapsed 4. Median number of chemo cycles before transplant: 2 (range 1-7). Previous autologous SCT: 3. Results. 4 patients received their alloSCT from a sibling donor, 10 from an haploidentical family donor or from an unmatch donor. Conditioning: 14 tr eosulfan+fludarabine±ATG, 1 donor, 10 from an haploidentical family donor or from an unmatch donor.

Discussion: prolonged OS is achievable with alloSCT in elderly pts with diagnosis of AML or poor risk MDS. AlloSCT should be offered to elderly pts selected according to PS and new available comorbidities score systems (e.g. Sorrow index). New treosulfan based conditioning regimen should allow successful transplant in these pts also from alternative donors. Adequate prevention and treatment of infections and effective GvHD prophylaxis could reduce the TRM and improve pts survival.

Figure 1. Massive hepatic damage.

Figure 2. Drug-induced necrotizing hepatitis.

Figure 3. Regeneration phase.

A severe clinical and laboratory picture of hepatic failure appeared. Given to this unusual course, a fine-needle biopsy of the liver was performed after plasma and platelet support. The histological examination showed a drug-induced necrotizing hepatitis (Figure 2). As the patient had a slow, spontaneous improvement of his clinical conditions, the treatment was restarted. After 6 R-CHOP21 he achieved a complete remission. Considering the very high risk of relapse, after mobilization with G-CSF he was treated with BEAM regimen and peripheral blood
stem cell support. Neither adverse events nor hepatic toxicity have been recorded and the patient is alive and well so far without evidence of disease. A subsequent CT showed features of hepatic regeneration confirmed by a second liver biopsy (Figures 3 and 4). The following considerations can be made from this case. First. We suppose the protracted exposition to acetaminophen as aetiological factor of liver damage, although a role of anaesthetic drug cannot be excluded. Second. Any radiological lesion in a patient with lymphoma has to be cautiously interpreted as localization outside the clinical context. Third. Serious but temporary organ impairment, per se, should not contraindicate the administration of high-dose chemotherapy.

Figure 4. Hepatic regeneration.

**PU-052**

THE OUTCOME OF A PATHOLOGICAL FRACTURE IN A MULTIPLE MYELOMA PATIENT DURING BORTEZOMIB-COMBINED THERAPY

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Myeloma bone disease is associated with excessive osteoclast activation and impaired osteoblast function. The increased osteoclast activity is considered to be mostly mediated through the activation of nuclear KB ligand/osteoprotegerin axis receptor. Recently it has been reported that myeloma cells can inhibit osteoblastogenesis through the block of osteoblast transcription factor RUNX2/CBFA1, indicating the role of myeloma cells in influencing the osteoblast formation and differentiation. Clinical data suggest that the proteasome inhibitor Bortezomib may increase osteoblast related markers such as osteocalcin and bone-specific alkaline phosphatase in multiple myeloma patients. A recent study have shown that Bortezomib significantly increases the transcription factor RUNX2/CBFA1 in human osteoblasts from multiple myeloma patients. These observations support the hypothesis that Bortezomib could have a direct stimulatory effect on bone formation process. The case history of a young female with multiple myeloma is presented. The patient was a 43-years-old woman was admitted in our Institute in December 2005 for multiple osteolytic lesions. Initial therapy with Talidomide and Dexamethasone was discontinued after two months for no response and for grade II neurological toxicities. Bisphosphonate at reduced dose were withheld after two cycles for persistent and severe hypocalcemia. A Vincristine, Adriamycin and Dexamethasone regimen was given for four cycles without any response and no improvement of her clinical condition. In July 2006 the patient experienced a pathological fracture of the right proximal humerus and impaired functional activity. She refused to undergo prosthetic replacement as treatment plan recommended by orthopaedic surgeon. A rescue therapy with Bortezomib 1.8 mg/mq day 1, 4, 8, 11, liposomal Doxorubicin 80 mg/mq day 1 and Dexamethasone 40 mg day 1-4 was initiated. After three cycles the bone marrow biopsy was hypercellular with 80% myeloma cells. Nevertheless the patient’s clinical conditions were improving. The patient achieved the resolution of bone pain at the right arm with functional recovery. This clinical observation was confirmed by the radiographic evaluation that showed evidence of a bone healing process at the site of humeral fracture; in addition the small osteolytic lesions spread to the humerus appeared less evident. At this time the patient is still on treatment and her clinical conditions have markedly improved. This case suggests that Bortezomib might have had a possible role in stimulating the bone healing process not linked to its antmyeloma activity. The patient did not receive bisphosphonate and no radiotherapy. Moreover, the improvement of the bone lesion to seem precede the reduction of marrow plasmacellular infiltration. The role of Bortezomib on bone lesions of multiple myeloma is very interesting and further clinical and biological data are need to better understand the clinical relevance of these preliminary findings.

**PU-053**

CASE REPORT OF A DELAYED HEMOLYTIC TRANSFUSION REACTION (DHTR) IN A PATIENT AFFECTED WITH MGUS

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Introduction. DHTR is due, in many cases, to anamnestic antibody production, but in some patients hemolysis results from the combination of high antibody levels and large numbers of transfused red cells in the circulation. Moreover a component of complement, activated by classical pathway or by fluid phase interaction, can attach to innocent bystander cells as well as the incompatible donor cells: this defines the reactive lysis. In this study we report a case recently observed. Methods. A female multipara patient, age 63, affected with a monoclonal gamopathy of uncertain significance (MGUS) was transfused with 5 units of (buffy-coat depleted) concentrate erythrocytes, AB0-Rh and cross-match compatible, during and just after a surgical treatment for aneurism of abdominal aorta. Results. During the post-surgical follow-up, the patient showed fever, pallor and mild jaundice. Laboratory data, retrospectively reviewed, revealed a post-transfusion hemoglobin (Hb) of 12.9 g/dl, but with a gradual drop, from 7th day, and a precipitous falling, on 10th day, to 5.1 g/dl. Suspecting a DHTR and obtained a patient’s fresh blood sample, we tested serum for unexpected alloantibodies and discovered two alloantibodies implicated and the most important in the course of the reaction. Discussion. Primary alloimmunization, demonstrable weeks or months after incompatible transfusion, is evidenced by appearance of newly formed antibodies to red cell antigens. Successfully, antibodies may diminish to undetectable levels, but with an anamnestic response if red cells that express the involved antigen are subsequently transfused. This response may cause the production, in hours or days, of IgG that react with the transfused red cells. In our case there are two alloantibodies implicated and the most important in the fixing of complement seems the anti-Jkb. Moreover, the total observed drop of Hb level was over 7 g/dl, while the complete hemolysis of 5 transfused blood units would generate a maximum theoretical drop equal to 5 g/dl; for this reason we can affirm that autologous erythrocytes were also hemolyzed as well as the donor cells. In conclusion, this report demonstrates the complement activation during DHTR and the hemolysis of innocent bystander autologous cells.
PU-054
POSTIRON EMISSION TOMOGRAPHY IDENTIFIES A DIFFERENTIAL PATTERN OF BONE MARROW FDG UPTAKE IN “POOR” AND “GOOD” PERIPHERAL STEM CELL MOBILIZERS


Objectives. The use of prophylactic G-CSF is associated to the increase of bone marrow (BM) fluorodeoxyglucose (FDG) uptake as detected by Positron Emission Tomography (PET). In contrast, no data is available as to changes in BM FDG-uptake during peripheral blood stem cell (PBSC) mobilization. This study was aimed at investigating patterns of BM FDG uptake during mobilization as quantified by Standardized Uptake Values (SUV). We also evaluated whether PET scanning may turn up of value for identifying good and poor mobilizers. To our knowledge, this is the first PET-based study in this setting. Methods. Seventeen patients (pts) (M/F=10/7), median age 51 yrs (28-65), with relapsed lymphoma (NHL/HD=13/4) without BM involvement, were accrued after informed consent. Baseline PET was obtained at relapse, before salvage therapy and any G-CSF administration. After salvage regimes, pts were mobilized by VRL/CTX or ARA-C; G-CSF (10 ug/kg/day) was given from d+6 through apheresis. PET scans were obtained on d+9 or +10 (after nadir with a WBC>1000/µL). SUVmax and average (avg) were measured (whole lumbar spine and bilateral iliac regions) and compared to SUV of the same BM regions at baseline PET. The aim was to calculate a BM specific Delta-SUV (mobilizing vs steady-state Delta-SUV) for each single patient. Results. Twelve pts mobilized PBSC (median CD34 peak 39.99/µL, r 22.8-280.58/µL; median CD34 in the harvest 3.3×10^4/Kg, r 2.1-12.5) while 5 pts were poor mobilizers (median CD34 peak 10.9/µL, r 7.5-14.1/µL). In the group of good mobilizers, apheresis was performed at CD34 peak (d+11,+14), with a median of 1 apheresis/pt (r 1-2). Unexpectedly, effective mobilization was associated with a low FDG-BM uptake: median BM Delta-SUVmax and Delta-SUVavg of 2.9 (r 1.0-3.8) and 2.2 (r 1.0-3.6), respectively. In contrast, poor mobilizers displayed a median Delta-SUVmax and Delta-SUVavg of 4.7 (r 2.4-12.8) and 5.9 (r 4.1-14.2), respectively. Conclusions. While FDG-BM uptake usually increases upon CFSC administration, our results suggest that PBSC mobilization may be associated with a complex metabolic pattern of BM as detected by PET. We documented that, 48-72hrs before CD34 peak, poor mobilizers display a higher FDG-BM uptake (Delta-SUV>3) as compared to good mobilizers (Delta-SUV<3). These preliminary results indicate that BM PET may represent a new tool for early identification of poor mobilizers allowing a timely modification of the mobilization strategy to possibly rescue the procedure.

PU-055
67KDA LAMININ RECEPTOR EXPRESSION IN HUMAN G-CSF MOBILIZED CD34+ PERIPHERAL BLOOD STEM CELLS CORRELATES WITH THEIR MOBILIZATION EFFICIENCY

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Introduction. The 67 kDa laminin receptor (67LR) is a non-integrin cell-surface receptor with high affinity for laminin which plays a role key in tumor invasion and metastasis. We investigated whether there was a modulation of 67LR expression in circulating CD34+ cells of normal subjects following G-CSF stimulation. Methods. Peripheral blood mononuclear cells (PBMC) were obtained from 40 healthy donors before G-CSF mobilization (day 0) and at various time-points during G-CSF administration (days 3-5). Phenotypic analysis of 67LR expressing CD34+ cells was determined by 3-color flow cytometry on a mononuclear gate of CD45+ cells. Results. G-CSF administration increased 67LR expression in circulating CD34+ cells; by contrast, unstimulated BM CD34+ cells showed very low levels of 67LR. Using as cut off a percentage + SEM of 67LR positive circulating CD34+ cells higher than 20%, 67LR expression resulted increased in 36/40 donors: mean percentage + SEM of 67LR positive circulating CD34+ cells 1.86±0.2% (range 0.5-7.2%) before G-CSF administration and 46.3±4.1% (range 23-56%) on the day of cell harvesting (p<0.0001). The mean percentage + SEM of 67LR positive unstimulated BM CD34+ cells from 15 normal subjects was 5.1±1.1% (range 1-13%). G-CSF withdrawal was associated with a rapid reduction of 67LR expression on CD34+ cells in all G-CSF-treated donors. Noteworthy, 4 out of 5 donors not showing 67LR increase on circulating CD34+ cells after G-CSF treatment were poor mobilizers; indeed, they obtained a peak of less than 20 CD34+ cells/µL and did not achieve the target CD34+ cell yield 2×10^6 CD34+ cells/kg in one apheresis procedure after 5 days of G-CSF administration. Accordingly, linear regression analysis showed that both numbers and percentages of 67LR positive circulating CD34+ cells after G-CSF administration directly correlated with CD34+ cell peak values on the day of collection (r=0.7, p=0.001 and r=0.5, p=0.002, respectively). Phenotypic analysis of 67LR expression in enriched CD34+/CD38- cells showed that the mean + SEM of 67LR expressing CD34+ cells at day 5 of G-CSF stimulation was 43±5% (range 36-60%), whereas it was 7.5±2% (range 4-10%), before G-CSF administration. Conclusions. Our data document that both numbers and percentages of 67LR positive circulating CD34+ cells after G-CSF administration correlate with the degree of CD34+ cell mobilization; indeed, poor mobilizing donors did not show 67LR increase in circulating CD34+ cells.

PU-056
18F-FDG PET AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN LYMPHOMA MALIGNANCES: A USEFUL TOOL TO GUIDE AND MONITOR POST-TRANSPLANT IMMUNOTHERAPY

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Introduction. Fluorine-18 fluorodeoxyglucose (“F-FDG) positron emission tomography (PET) in lymphoma patients after allogeneic stem cell transplantation (AlloSCT) has been explored and described in only one published report. PET monitoring of the disease status after AlloSCT proved useful in managing post-transplant immunomodulation. The objective of our study is to review the experience with PET after AlloSCT in our pts with lymphoid malignancies, to assess its value in guiding adoptive immunotherapy with donor lymphocyte infusions (DLI). Methods. We retrospectively reviewed PET results in 13 pts during follow-up after AlloSCT for refractory or relapsed Hodgkin’s (n=1) and non-Hodgkin’s lymphoma (n=12), at our Institution. Median number of therapy lines before AlloSCT was 2 (range 1-4). Conditioning was myeloablative in 6 patients and reduced-intensity in 7 patients. A total of 79 PET scans were performed with a median of 5 PET scans per patient. Pts with a negative PET after AlloSCT went on with follow-up while those who showed PET-positivity received DLI; chemotherapy was administered before DLI in 3 cases, after clinical decision according to the disease status detected. Results. 1/13 Pts with a negative PET repeatedly negative in 7 pts, 5 are alive in complete remission with a median follow-up of 77 months (range 16-86), 2 died without disease recurrence after 46 months, for a second malignancy, and 11 months, for cGVHD, respectively. In 6 pts PET was positive, after a median of 5 months (range 2-6) after AlloSCT, leading to immunosuppression withdrawal and to DLI (1 patient), rituximab and DLI (1 patient), rituximab and DLI after chemotherapy (3 pts), rituximab and DLI followed by IL-2 and interferon (1 pt). In these pts a complete remission was achieved in 4 pts with no response or progressive disease, (all pts died), in 2 cases a documented response to adoptive immunotherapy (Graft-versus-Lymphoma effect); these 2 pts are alive and progression-free at 22 and 26 months after AlloSCT, respectively. Discussion. This retrospective study suggests the favourable high predictive value of PET negativity in patients with lymphoid malignancies after AlloSCT. Moreover, PET proved useful to monitor and to detect early disease relapse or progression during follow-up, guiding DLI-based immunotherapies, which are more effective when disease burden is limited. More cases are warranted to confirm our data.

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ENHANCED BONE MARROW HARVESTS FROM ALLOGENIC HLA-ID SIBLINGS DONORS RESULT AND IN CD34+ CONTENT AND NEUTROPHIL ENGRAFTMENT TIME EQUIVALENT TO PBSC SOURCE AND IN A LOW TRM.

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Introduction. Number of infused HSC is considered to have an important effect after BMT on patient’s survival, patients receiving a number of CD34+ cells below median having a lower survival. In order to increase the median number of CD34+ cells infused and to improve survival we have optimized our bone marrow harvest technique. Aim of our study was to compare characteristics of inoculum, engraftment times and TRM after allogeneic transplants using Enhanced Bone Marrow or PBSC harvest. Methods. A group of 65 consecutive allogeneic patients was studied. Patients received HLA identical transplant from sibling for various malignant diseases; assignment to either source group was decided based on disease status and on donor sex; 36 pts received BM and 29 PBSC. Optimization of BM Harvests was obtained using large and multi-bore needle (11 g), small volume of aspiration (3-5 mL) and allowing only an experienced physician to perform the procedure. PBSC were mobilized with G-CSF 10 mcg/kg/day with a target infusion dose of 5×10^9/Kg. Median donor weight was 74.5 Kg in BM group and it was 71.2 Kg in PBSC group (t-test: p=0.6). GVHD prophylaxis was done in both groups with CSA plus standard dose MTX and number of MTX days was comparable in both groups with CSA plus standard dose MTX and number of MTX days was comparable in both groups. A count of PLT> 50.000 was reached at 15 days) in respect to BM (median at 18 days) (log rank: p=0.1). Engraftment time to N.×10^9/L, was comparable in the two groups: 17.8 days in BM and 18.0 in PBSC group (log rank: p=0.4), no patients received G-CSF administration. A count of PLT> 50.000 was reached faster in PBSC group (median at 15 days) versus BM group (median at 18 days) (log rank: p=0.04). In patients transplanted in early phase of their disease, OS was 80% in BM group and 70% in PBSC group (log rank: p=0.4) while in advanced phase patients OS was significantly better after BM transplantation in respect to PBSC (65% versus 20%; log rank: p=0.01), TRM at 1 year was 4.7% in BM group while it was 27.3% in PBSC (estimated by KM). Discussion. In conclusion enhanced BM harvest results in a CD34 content and myeloid engraftment time equivalent to PBSC and in a low TRM.

A CASE OF PRIMITIVE LARGE B-CELL TESTICULAR NHL IN MULTIPLE MYELOMA.

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We report a case of multiple myeloma (MM) in remission after Bortezomib (BTZ) treatment that developed high grade testicular non-Hodgkin lymphoma. Case Report. Man, 77 years old, in observation for a monoclonal component (MC) IgG(K). He came to our institution in 11/04 because of hypercalcemia and increasing monoclonal component (gr 5.0%). Bone marrow aspiration showed 70% of plasma cell infiltration. Because of kidney insufficiency, hypercalcemia and anemia, treatment with pamidronate, hyperidratation and MP was started. Cycles were repeated every four weeks until partial remission. In 8/02 because increasing of MC, CTX+DMZ every 4 weeks, was started. After six cycles treatment with thalidomide 100 mg/day was started. Patient prolonged this dosage until 12/08 when peripheral neuropathy appeared and dosage was tapered to 100 mg for 10 days a month. After two years was observed progressive anemization was observed and bone marrow biopsy revealed 36% of plasma cells infiltration with 6.5% of cells with 13q14 deletion. We started BTZ+DMZ every 21 days. After six cycles a reduction of MC without signs of plasma cells infiltration was reached. 2 months after, a progressive testicular tumefaction appeared. After echotomography, TC, RMN and failure of antibiotic therapy transinguinal orchietomy was performed. Testicular histology showed diffuse infiltration of testis by large lymphoid cells with evident cytoplasm and vesicular nucleus with prominent central nucleolus. Areas of necrosis were present. Neoplasia infiltrated albignea and initial portion of spermatogenic structure. Immunochemistry examination showed positivity of lymphoid cells for CD20, CD79a, Bcl2, partially positivity for Bcl6 and negativity for CD3, CD5, CD10, CD30, CD138, EMA. Cyclant cells...
was used. The conditioning regi-
men to an allogeneic hematopoietic stem cell transplantation with this pro-
tocol of treatment. HLA identical sibling donors. Median patient age was 40 years (r.25-58).
The stem cell source was unmanipulated peripheral blood in all cases.

### Table 1.

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<th>OB</th>
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<td>Fever</td>
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<td>Organ dysfunction</td>
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The median follow-up after transplant was 14.6 months (r.2.4-34.5) and 10 months (1-12) for OB and IB group, while PFS was 13.6 months (10-16) and 10 months (1-12), respectively (p=ns). Finally, in term of costs the complete course of Busilvex is significant higher than the
Oral mucositis is a well-known, potentially devastating side effect of intensive chemotherapy and currently no standard therapy is available to prevent severe oral mucositis. We tested the efficacy of Palifermin (recombinant human keratinocyte growth factor) to decrease severe oral mucositis induced by high dose chemotherapy conditioning for autologous stem cell transplantation. Since October 2005 to September 2006, in the course of an Amgen multicentric trial, 15 patients (12 male, 3 female; median age 49, range 19-65) with haematologic cancers (4 with Multiple Myeloma, 2 with Acute Myeloid Leukemia, 2 with Hodgkin disease and 7 with Non Hodgkin Lymphoma) received Palifermin (60 micrograms per kilogram of body weight per day intravenously) for 3 consecutive days immediately before the initiation of conditioning therapy and after autologous stem cell transplantation. Oral mucositis was evaluated daily for 28 days after transplantation using the WHO oral toxicity scale. Conditioning therapy consisted of melphalan 200 milligrams/metrosquare for patients with multiple myeloma, busulfan 16 milligrams/kilogram and cyclophosphamide 120 milligrams/Kilogram for patients with acute myeloid leukemia, BCNU 300 milligrams/metrosquare, etoposide 400 milligrams/metrosquare, ara-C 800 milligrams/metrosquare, melphalan 140 milligrams/metrosquare for patients with Hodgkin and non Hodgkin lymphoma. The main adverse events were rash (20%), pruritus (6%), taste alteration (33%), transient increase of serum amylose (6%) and skin ikerpigmentation (6%). All these events were transient and mild in severity, only in one case led to the discontinuation of the drug. Among the 15 patients treated with Palifermin, 11 had no mucositis at all, 2 had an oral mucositis of grade 1 and 2 patients presented an oral mucositis of grade 2. The duration of mucositis in those patients was 4 days. None developed WHO grade higher than 2 oral mucositis, none required parenteral nutrition or opioid medication. Then we compared the incidence and severity of oral mucositis in a matched control group of 30 patients (Ratio 1:2) with the same characteristics in terms of median age, type of disease and conditioning therapy who undergone autologous stem cell transplantation from November 2004 to December 2006 not receiving Palifermin. Among these patients, 9 had an oral mucositis of grade 3, 7 had an oral mucositis of grade 2, 8 had no mucositis at all (p=0.05). Among these patients 2 required parenteral nutrition and 8 opioid analgesics. Palifermin reduced the incidence and severity of oral mucositis in patients candidate to high dose chemotherapy and autologous stem cell transplantation.

PU-065
Efficacy of high-dose therapy followed by autologous stem cell transplantation in lymphomas: the Cagliari experience
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Autologous stem cell transplantation has proven to be an effective approach in selected patients with Hodgkin’s (HL) and non Hodgkin’s lymphoma (NHL). The purpose of this study was to evaluate the long term outcome of high dose therapy followed by autologous stem cell transplantation in 77 patients affected by lymphomas treated in our institution. From January 2003 to December 2006 57 consecutive patients with NHL (median age 49, range 22-64) and 20 consecutive patients with HL (median age 59, range 20-61) were treated in our Centre with myeloablative therapy followed by autologous stem cell transplantation. Among the patients with NHL, 44 (77.1%) were diagnosed with Diffuse Large B Cell Lymphoma, 5 (8.7%) with follicular lymphoma, 3 (5.2%) with mantle cell lymphoma, 3 (5.2%) with central nervous system lymphoma and 2 (3.5%) with MALT lymphoma. The majority of patients (63%) had advanced disease (17 stage III, 19 stage IV of Ann Arbor classification); 31 (54%) had received first line therapy and 26 (46%) up to 3 lines of conventional chemotherapy before autologous transplant. Fifty-four of the 57 patients with NHL received BEAM (BCNU, Etoposide, Cytarabine, Melphalan) as conditioning regimen; the 3 patients with central nervous system lymphoma received BET (BCNU, Etoposide, Thiotepa). Median CD34+ cell number for transplantation was 2.5x10^5 per kilogram of body weight, median time for platelet recovery (> 20 000/microliter) and for leukocyte recovery (> 500/microliter) were 12 and 11 days, respectively. With a median follow up of 30 months (range 3-62), 46 of the 57 NHL patients (80%) are alive and 11 (19%) died, 2 for Transplant Related Mortality (TRM), 7 for progression of disease and 2 for other transplant not related causes. Among the 46 alive patients, 42 (91%) are in complete remission, 2 (4,3%) had a relapse and 2 (4,3%) obtained a partial remission. Among the 42 NHL patients who achieved a complete remission after transplantation, 18 (42%) were in complete remission even before the transplant, 24 (57%) were in partial remission. Among the 11 NHL relapsed patients, 10 (90%) were in partial remission before the transplant and one was in complete remission. Among these patients median disease free survival was 12 months (range 3-48). The 2 NHL patients who obtained a partial remission had the same pre-transplant status. Among the 20 HL patients, 12 (60%) had advanced disease (7 stage III, 5 stage IV of Ann Arbor classification). All of them achieved a complete remission and received autologous stem cell transplantation before autologous transplant. Conditioning regimen administered was BEAM. With a median follow up of 30 months (range 3-62), 13 of the 20 HL patients (65%) are alive, 7 (35%) died, all of them for disease progression. Autologous stem cell transplantation resulted in a complete remission in 7 patients (35%) and in a partial remission in 2 patients (10%). Eleven patients had a relapse (55%). Among the 7 HL patients who achieved a complete remission after transplantation, 2 (28%) were in complete remission even before, 5 (70%) were in partial remission. Among the 11 HL relapsed patients, only 1 was in complete remission before the transplant, 10 were in partial remission. Among these patients median disease free survival was 3 months (range 2-24). Our results confirm the efficacy of high dose therapy followed by autologous stem cell transplantation in patients affected by Hodgkin and non Hodgkin lymphomas. The incidence of relapse is higher in the HL group probably because most of these patients underwent high dose therapy after at least 2 or 3 relapses with conventional chemotherapy and many of them were not in complete remission at the time of the transplant. However in this group of patients autologous transplant appears to increase disease free survival. Better results were obtained by the NHL group in which the majority of patients (73%) achieved a complete remission after high dose therapy and the incidence of relapses is lower (19%) and occurred in most cases (90%) in patients who were not in complete remission at the time of the transplant. Therefore status pre-transplant seems to be an important prognostic factor for the outcome of this therapeutic approach.

PU-066
Hematopoietic stem cell transplantation after reduced intensity conditioning regimen (RIC-HSCT) in advanced hematologic malignancies: the Cagliari experience
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Reduced intensity conditioning regimens have been used in the recent years with the aim to decrease transplant related mortality (TRM) and to extend this procedure to a wider patient population. We report a retrospective study concerning 22 patients who underwent RIC-HSCT for hematologic and non-hematologic malignancies (6 Females, 16 Males, median age 30 years, range 20-64), between March 2002 and March 2006. Diagnosis were: 2 AML, 4 MDS, 2 CLL, 3 NHL, 2HD, 2 IMF, 5 MM, 1 SAA, 1 metastatic colon cancer; all patients had advanced heavily treated disease. At transplant 4 patients were in first remission (CR), 1 in partial remission (PR) and 17 in progressive disease (PD). Peripheral blood stem cells (PBSCs) were used in 14 patients and bone marrow in 8, all derived from HLA identical related donors. As conditioning all patients received Thiopeta based regimens in association with
Fludarabine. Cyclophosphamide and melphalan according to their primary disease. As GVHD prophylaxis all patients received Cyclosporine A (CSA) and sMTX. After transplant 5 patients developed Acute GVhd (4 ex novo, 1 after DLI); it was grade I in 3 and grade III in 2. Chronic GVhd occurred in 6 patients; it was limited in 5, severe in 1. Twelve (12) out of 22 patients (54%) died in this group (6 for relapse or progression disease, 6 for TRM), 10 pts are alive (5 in CR, 5 with the disease). With a median follow up of 21 months, the 4-year probability of overall survival (OS) and event-free survival (EFS) are 55% and 21% respectively. This limited experience underlines that RIC-HSCT may be a treatment option for a subgroup of high risk or older patients but a still high rate of failure persists due to the recurrence of the underlying disease. Disease status at transplant remains the most important risk factor for outcome.

PU-067
ACUTE LEUKEMIAS AND MOLECULAR MARKERS COOPERATIVE STUDY BETWEEN CANCER HOSPITAL A. BUSINCO Cagliari and CANCER INSTITUTE S.O.L.C.A. of CUENCA IN (EC)

Culurgioni F; Liliu S, Pala G, Cardia D, Zucca MG, Sedda G, Ennas MG, Angelucci E

Unità Operativa di Ematologia e Centro Trapianti Muldel Osso, Ospedale Oncologico A. Businco, Asl 8 Cagliari, Italy

Introduction. This molecular study on acute leukemias is the result of the collaboration between Department of Haematology and Transplant of Cancer Hospital Businco, of Cagliari(I) and Molecular laboratory of Cancer Institute S.O.L.C.A. of Cuenca (EC). Prognosis, diagnosis and follow up in children and adults with Acute leukemias with has been stud-
ied. Source of Ecuadorian samples were patients who come from Azuay countries, Loya, El Oro, Rio Paute. Countries with high deforestation tax, few health services, high use in agriculture of chemicals associated with high poverty level and high incidence of chronic disease in blood, pneumonia, breast. Ecuadorian population studied is included in a geographic area of 700,000 km², 8% were Indians, 0.25% negro, 94% half-caste white. Ecuadorian incidence of Acute lymphoproliferative Leukemias was 3.0 /year/100.000 people; AML 1.4/year/100.000 people. High frequency were observed in children (0-14 years) with a 66% of tumor deaths. Among ALL pediatric case, 80% were type B, among B-ALL 72% case of adults were type L2 and 75% of children type L1 according to FAB classification. Molecular analysis were performed in Cuenca(EC) and only samples of positive case were sent in molecular Biology of Cagliari for confirmation of results and sequence analysis. Objective. To create a new molecular laboratory for ALL/AML diagnosis in Cuenca(EC) was the aim of the study. Materials. From February 2000 to February 2005 in Cancer Institute S.O.L.C.A. of Cuenca (EC) 261 cases (153 male, 124 female) with lymphoproliferative disease,119 of them were acute leukemias (38 AML and 81 ALL) were analyzed. Methods. All cases were analyzed with Polymerase chain reaction, BIOMED 1 PCR protocol were performed to value t(1;19), t(9;22), t(8;21), t(15;17), t(12;21) rearrangements, while for MLL rare translocations study t(6;11),t(9;11) t(11;19) were used been probes and procedure kindly provided from Instituts fur Pharmazeutische Biologie JWG Universitaet Frankfurt. Results. The 15% of patients showed genetic alterations (12% in adults and 14% in children), 1% of children were positive to 1 Chromosome deletion. In ALL, 1 case (1.23%) was positive to t(4;11) rearrangement, all results were tested with BIOMED 1 protocol and confirmed with sequence analysis. Considerations and conclusions. Molecular Results of patients in Cuenca(EC) were confirmed with results of same samples sent to Cagliari. To our collaboration, after 6 years the molecular labo-
ratory of Cancer Institute S.O.L.C.A. of Cuenca (EC) is an independent structure for molecular diagnosis in leukemia, lymphomas, transplant and cordonal blood procedure.

PU-068
COMPLETE REMISSION OF A PRIMARY CUTANEOUS DIFFUSE LARGE B- CELL LYMPHOMA (PCDLBCL), LEG TYPE IN ELDERLY PATIENT BY MONOTHERAPY WITH RITUXIMAB

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1’U.O.S. di Onco - Ematologia; 2’U.O. di Oncologia, O.C. A. Cardarelli, ASREM, Campobasso, Italy

The primary cutaneous diffuse large B cell lymphoma of the leg is an uncommon subset of pcDLBCL (about 10%), aggressive, that generally hits the legs of elderly women with plates and/or nodules and that has a rapid growth, a greater tendency to the dissemination and unfavorable prognosis. Histopathologic examination of the lymph nodes showed dense lymphocytic infiltrates with predominantly of large dysplastic B lymphocytes cells CD20+ and component reactive T absent. The immunophenotype of cells are CD20+, CD79a, bcl2+, CD10+, bcl6 –, MUM-1/IRF4+. Generally the pcDLBCL is not accompanied by the translocation t(14;18). The survival to 5 years is of 50-60%. The prog-
nosis is influenced negatively by the presence of multiple lesions. The therapy of choice is the chemo therapy with CHOP like protocols; the radiotherapy can be utilized for the patients with single lesion. The rit-
uximab therapy alone or in combination to the chemotherapy is a valid alternative. We report a case of 84-year-old, multimorbid woman who was affected by such a non - resectable large B-cell lymphoma, leg type variant, disseminate to the skin of the forehead. The patient introduced to the forehead nodules to clusters, easy bleeding of the diameter of 1-2 cm, red with most numerous plates satellites on a bottom erythe-
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The purging effect of Rituximab during autologous peripheral blood stem cell transplantation (APBSCT) was explored in a cohort of adult patients affected by relapsed or high-risk non-Hodgkin lymphoma. The study aimed to evaluate the safety and feasibility of a concurrent administration of Rituximab with high-dose chemotherapy to achieve complete remission (CR) in patients with diffuselymphoma refractory to ASCT. Rituximab was given intravenously on the day before and +7 of the treatment. Mobilising regimen was also utilized to enhance the stem cell harvest. Patients received a median of 3 red blood cell transfusions and 2 platelet transfusions. No patient died of treatment-related mortality. The median time to relapse was 5 months, and 5 patients relapsed. The OS and DFS were 84.4% and 66.5% at 12 months. Longer follow-up is needed to evaluate any eventual advantage in terms of OS and DFS compared to previous published studies.

<table>
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<th>Table 1. Demographic data.</th>
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<td><strong>Gender</strong></td>
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<td><strong>Platelet transfusions</strong></td>
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Figure 1. IgG level baseline, 6 and 12 months after ASCT.

Figure 2. Overall survival and disease free survival.
 PU-072
EXTRAMEDULLARY RELAPSE OF MULTIPLE MYELOMA AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION WITH SHIFT OF SERUM MONOCLONAL COMPONENT

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Introduction. Extramedullary manifestations with or without minimal monoclonal component (MC) has been reported in about 15% of multiple myeloma (MM) patients after autologous peripheral blood stem cell (PBSC) transplantation. Bortezomib is an effective agent in MM patients with extramedullary disease. Recently, we have shown a high frequency in extramedullary disease (EMD). Aim. We are reporting the case of a patient with IgG MM who had had, after autologous PBSC transplantation, IgD extramedullary relapse sensitive to treatment with bortezomib. Case report. A 48-year-old man was diagnosed with IgG-lambdamma MM in August 2000. He was treated with 4 cycles of VAD (vincristine, doxorubicin, dexamethasone) chemotherapy and with melphalan 200 mg/m² and autologous PBSC transplantation achieving a complete response (CR) which was maintained with subcutaneous alpha-2b-interferon 3MU/3d weekly. In November 2005 he presented a pericardial mass (8 x 4 cm) evaluated by chest radiography, computed tomography (CT) scan and by echocardiogram. The surgical operation removed the pericardial mass. Histological examination showed diffuse monoclonal lambda plasma cells infiltration. Serum protein electrophoresis showed a small (0.4 g/dL) paraprotein in beta region and serum immunofixation revealed IgD MC. Bone marrow aspiration showed <10% plasma cells and skeletal X-ray survey showed the absence of osteolytic lesions. In December 2006 the patient started treatment with bortezomib at the dose of 1.3 mg/m² on days 1, 4, 8, 11 plus dexamethasone at dose of 20 mg on days 1,2, 4, 5, 8, 9, 11, 12 every 21 days. After 4 cycles CR was obtained with negative serum immunofixation and absence of other clinical signs of disease. Discussion. The pattern of relapse of MM after autologous PBSC transplantation is very heterogeneous. EMD at relapse is relatively frequent (about 15% of cases) generally with the same MC presented at diagnosis. In our case the EMD presented associated with shift of MC. The different clinical and laboratory expression of relapse may be due to clonal selection after high-dose therapy and could indicate the persistence of a resistant clone. Moreover the effectiveness of bortezomib in case of EMD relapse, probably due to extensive tissue penetration, is confirmed by our experience.

 PU-073
IMMUNO-SUPPRESSIVE HIGH DOSE THERAPY AND AUTO-TRANSPLANTATION IN A CASE OF RESISTANT THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

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The thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy clinically characterized from: thrombocytopenia; haemolytic anaemia; neurological deficit; renal insufficiency and fever. The plasma exchange (PE), in association with steroids, it currently represents the first-line treatment. Among 11% and 36% of the patients present recurrent episodes of TTP, in a majority of the cases it have shown a severe deficit of the metalloprotease ADAMTS13 correlated to an inhibition of nature anti-body. The high doses immunosuppressive therapy (HDIT), with or without autologous transplantation (ASCT), has been used in different autoimmune disease resistant for, with the immunological reset followed by high doses therapy, to destroy the clones or oligoclones population of lymphocyte auto-reagents and to restore a functionality immunity normal. The experience in the TTP with this treatment is limited. 47 year-old woman with recurrent episodes of TTP treated with HDIT and auto-transplant. The patient has presented among 1999 and 2004, 6 episodes of TTP, initially treated with infusion of plasma, steroids and vincristine, subsequently with PEP and steroids. The remission of the TTP more than one year has not lasted and the last episode in August 2004 has poor responded to the treatment and has exposed the patient to serious danger of life. In September 2004, after mobilization with high doses of cyclophosphamide (7 g/m2) the stem cells have been harvests (4x10^9/kg) and in December 2004, after conditioning with Fludarabine (50 mg/m2 from the day -6 a day -4); cyclophosphamide (60 mg/Kg day -3); G-CSF (from day +1) and mycophenolate mofetil (600 mg/m² from day +6), the patient has been auto-transplanted. Not has been shown fever in aplasia and the patient has not had necessity of transfusion. The attachment has been documented a day +8 after transplantation. The treatment with mycophenolate mofetil has been continued for two years. Currently the patient is +50 months from the transplantation and has not had new episodes of TTP. The association HDIT/ASCT has been used to the purpose to eliminate the auto-immunity in the patient through the conditioning and to have a rapidly haematological recovery with the infusion of stem cells. The use prolonged of mycophenolate mofetil has served to control the new immunological order restored after such treatment. Therapy has excellently been supported, have not been documented side effects and the patient has had the longest period of remission. In conclusion such procedure can be an option for the treatment of the patients with resistant autoimmune disease and not curable, also after treatment with rituximab.

 PU-074
SEQUENTIAL THERAPY OF RITUXIMAB AND THALIDOMIDE IN A CASE OF MULTICENTRIC CASTELMAN'S DISEASE

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Castleman’s disease (CD) was first defined in 1956 by Castleman. Thrombocytopenia manifestations have been developed (byline vascular variant, plasma cell variant, and mixed variant) and two clinical classifications (unicentric [unifocal or localized] and multicentric [multifocal or generalized]). The multicentric presentation is often composed of lymph nodes with the plasma cell or mixed pathology. This presentation requires systemic therapy and prognosis is guarded. Associated systemic symptoms are common. There is an increased incidence of CD in patients with HIV. The human herpes virus-8 is associated with nearly all of the HIV-associated CD cases and nearly 50% of non-HIV cases. Interleukin (IL)-6 has also been shown to play a significant role in the pathogenesis of the disease. We describe a case of multicentric (plasma cells variant) CD, not HIV and HHSV8 correlated, has been treated sequentially with rituximab and thalidomide. 53 year-old male, has been hospitalized in the August 2006 for fever, systemic symptoms, anaemia and pentoneal effusion. A TC has shown limpho-megaly in abdomen (max 4 cm of diameter) and hepato-splenomegaly. For the bad and serious clinical conditions the patient has effected 2 operations (in laparoscopy and laparotomy) for the biopsy of the lymph node for to define the diagnosis of CD plasma-cells variant. In September 2006, for to persist of the fever and of the bad general conditions waiting for the histology, the patient has begun treatment with high doses of steroids and not specific immune-globulins with modest improvement of the general conditions and the general symptoms. After the histological restoration of the diagnosis needed for LMP to fulfill the criteria, the patient has been treated with a combination chemotherapy, combination chemotherapy, interferon (IFN)-alpha, rituximab, anti-IL-6 receptor antibodies, and thalidomide. This disease, often, hides an aggressive lymphoma. Today the new drugs allow a not aggressive therapy and many active. In literature, cases have been signalled treated with success with rituximab single agent (patient positive HIV). While the results with the alone thalidomide are doubtful. This is the first case where a sequential therapy has been effected with rituximab single agent (patient positive HIV).
PU-075
ACUTE MYELOID LEUKEMIA IN THE ELDERLY, THE INTENSIVE CHEMOTHERAPY IT IS NOT SUPERIOR OF LOW DOSE THERAPY AND/OR MAINTENANCE. OUR EXPERIENCE IN PATIENTS OVER 65 YEARS
Pezzullo L, Finizio O, Rocco S, Bene L, Nunziata G, Ferrara MG, De Rosa C, Buonanno MT, Mettivier L, Mettivier V
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The treatment of acute myeloid leukaemia in elderly with age > 65 years is still debated. In literature numerous studies have valued the feasibility of intensive chemotherapy in these patients. The aim of the study is to value the difference in EFS and OS among 2 groups of AML elderly patients treated with intensive chemotherapy (IC) or maintenance (M). From June 2001 to May 2007 we have treated in our Division 66 AML patients, 37 male and 29 female with median age of 73 years (66-90 years). 31 patients (18 M and 13 F with median age of 69,5 years) have received intensive chemotherapy (IC. Flag and MICE) and 35 (20 M and 15 F with median age of 79 years) have received maintenance (low dose cytarabine and/or support). In IC group 16 patients (52%) have obtained complete remission (CR) with to EFS and OS media of 5, 3 and 8, 6 months respectively, the rate of TRM has been of 28%. In the M group the CR has been documented in 9 patients (55%) with to EFS and OS media of 4,8 and 5,6 months respectively (Figures 1-2).

Figure 1.

This results have shown a best rate of CR in the IC group but the OS (p=0.6) and EFS (p=0.9) difference is not statistically significant in the two groups. In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients. New therapeutic strategy is necessary for to improve the EFS and OS in these patients. Interesting is the use of new drugs as: humanized anti-CD33 antibody (gemtuzumab ozogamicin), tyrosine kinase inhibitors, 5-azacytidine (vidaza) and 5-aza-2’-deoxycytidine (decitabineTM; dacogen), multidrug resistance inhibitors, farnesyl transferase inhibitors, histone deacetylase and proteosome inhibitors, antiangiogenesis agents, FLT3 and antiapoptosis inhibitors, are all options under investigation, in this poor disease especially in maintenance after a CR obtainable with an intensive or low dose chemotherapy.

PU-076
COMPLETE AND PROLONGED REMISSION OF LINFOPLOSMOCYTIC LYMPHOMA AND REPRESSION OF ASSOCIATED SEVERE RENAL FAILURE AFTER TREATMENT WITH BORTEZOMIB
Monaco G, Ligouri L, Focarelle E, Iaccaroni S, Troiano M, Abbassasa A
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Background. We describe a case of lymphoplasmocytic lymphoma, refractory to conventional treatments, complicated with bone lesions and acute renal failure, completely regressed after therapy with bortezomib. Case report. Woman, 48 years old, in march 1994 receives diagnosis of lymphoplasmocytic lymphoma, refractory to conventional chemotherapy (chlorambucil, CHOP, fludarabine), immunotherapy (rituximab), radiotherapy. She comes to our attention in november 2005 for disease’s progression, associated to severe renal failure (seric creatinina=1,7 mg/dL; creatinine clearance=28,86 ml/min) because of severe neurologoc toxicity (convulsion, peripheral neuropathy) and appearance of herpetic lesions, we decide to suspend treatment and bortezomib. After eleven months from the end of treatment with bortezomib, PET total-body, shows only a moderate hypercaptation on the anterior segment of lower lobe of right lung. At a follow-up of seventeen months renal function results clearly improved (seric creatinina=1,4 mg/dL; creatinine clearance=44,57 ml/min) with normalization of proteinuria (104 mg/24h).

Discussion. Renal failure in course of lymphoplasmocytic lymphoma is a not frequent event. It is due to amount of paraprotein on endothelial side of glomerular basal membrane. Generally standard treatments not improves the renal failure. The therapeutic activity of the antiproteosome in myeloma and in different lymphomas is currently well documented (O’Connor et al., JCO 2005, 23 (4):676-684; K. Anderson et al, JCO 2006 Annual Meeting Proceedings Part I. Vol 24 (18S): 7504). Others autors have demonstrated the renal repair (A. K. Malan et al., Acta Haematologica 2006;116 (4):255-258). Conclusions. Our case shows the therapeutic activity of the antiproteosome, bortezomib, can be used to improve not only myeloma and lymphoma course but also to repair renal failure, when caused by paraproteinemia/paraproteinuria.

PU-077
COMPLETE REMISSION AFTER INFUSION OF PERIPHERAL BLOOD STEM CELLS IN CHEMOTHERAPY-REFRACTORY ACUTE LYMPHOID LEUKAEMIA DURING APLASTIC PHASE WITH BLASTIC BONE MARROW: REPORT OF TWO CASES
Rocco S, Pezzullo L, Finizio O, Martorelli MC, Ferrara MG, Buonanno M, Mettivier V
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Relapse is a common feature in ALL and is often refractory to chemotherapy, leading in prolonged aplasia, infections, transfusion requirement and keep out patients to new treatments. Here we report 2 consecutive cases in which infusion of previously collected peripheral stem blood cells (FBSC) during aplastic phase post-chemotherapy due to leukemic infiltration of bone marrow has been followed by haematologico reconstitution and morphological and immunophenotypic remission of the disease. Case n. 1. Patient aged 22 years, male. On day 35 was still pancytopenic and acute renal failure, completely regressed after therapy with bortezomib, there is a conspicuous reduction of proteinuria (350 mg/24h) with partial recovery of renal function (seric creatinina=1,7 mg/dL; creatinine clearance=28,86 ml/min) because of severe neurologoc toxicity (convulsion, peripheral neuropathy) and appearance of herpetic lesions, there is a conspicuous reduction of proteinuria (350 mg/24h) with partial recovery of renal function (seric creatinina=1,7 mg/dL; creatinine clearance=28,86 ml/min)

Case n.2. Patient aged 36 years, male. Standard risk ALL relapsed two years after diagnosis, treated with high dose ara-c and mitoxantron. On day 35 was still pancytopenic and acute renal failure, completely regressed after therapy with bortezomib, there is a conspicuous reduction of proteinuria (350 mg/24h) with partial recovery of renal function (seric creatinina=1,7 mg/dL; creatinine clearance=28,86 ml/min)
SUCCESSFUL MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS WITH USE OF GRANULOCYTE-COLONY STIMULATING FACTOR AND R-CHOP REGIMEN: OUR PRELIMINARY EXPERIENCE
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Introduction. Actually more diffused method for mobilization of peripheral blood stem cells (PBSC) consists in the employment of the chemotherapeutic regimen plus granulocyte colony-stimulating factor (G-CSF). The mobilization of the PBSC from the peripheral blood depends also from effects of the previous treatments. For the mobilization of PBSC in non Hodgkin’s lymphoma patients (LnH) are employed several chemotherapeutic regimens and drugs. Our study analyzes the mobilization of PBSC following CHOP plus rituximab (R-CHOP) therapy in patients with LnH. Methods and Results. From January 2006 to today we have analyzed leukapheresis for PBSC in seven patients with LnH after treatment with R-CHOP regimen + G-CSF (filgrastim). Probably the results are without interest for the accredited hematologic transplant’s centres but it has a great importance for our department because we have only recently begun the ASCT program. We mobilized PBSC after R-CHOP regimen in seven patients with LnH (2 males and 5 large cells; two patients were in IV stage for bone marrow involvement; the mean age was 43 years; range 29.55 years). All patients were at first treatment for LnH and at the time of PBSC mobilization were in partial remission and at fourth or fifth cycle of R-CHOP. All patients were given G-CSF at dose 5 micrograms/Kg starting from day 4 after R-CHOP therapy. The CD 34+ blood cell count were performed before of the cycle of chemotherapy, before the beginning of the G-CSF at day 4 and subsequently daily. The increase of the CD 34 have daily been progressive with doubling then of the value around the 10-11° day post R-CHOP. The single leukapheresis was performed in all patients when the values of cells CD34+ were over 20/microlitres. The leukapheresis product was always over 5 cells CD34×10^9/Kg. In the same period we have appraised besides, without however to submit them at leukapheresis for refusal, other 3 patient with LNH with same results. Conclusion. The combination R-CHOP + filgrastim (at 5 μg/Kg/once day) in non Hodgkin’s patients has a good effect of mobilization of the PBSC and of course offers the possibility to recover the PBSC with the same standard treatment for the LnH, premise to economize and also is feasible as precautionary procedure for refractory or relapse of disease. The yield of leukapheresis was always more than 5 cells CD34×10^9/Kg.

WHAT IS THE BEST CONDITIONING FOR AUTOTRANSPLANT IN AML PATIENTS?
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The conditioning regime more used in bone marrow transplantation, in patient with AML, has been the BuCy-2. Such treatment, mieloablative and immunosuppressive, he favours the engraftment of the marrow in the allogeneic transplant, not clear the advantage in the autologous transplant. In the last years, new conditioning regimes to more intense mieloablation has been used in AML autotransplant patients. We have retrospectively evaluated, in terms of efficacy and results, the classical conditioning BuCy-2 with a conditioning with busulfan (4 mg/kg days -5,-3) and melphalan (140 mg/m² days -2) (BuMel). From June 2001 to December 2006 we have autotransplanted 30 patients with AML in first CR (11 males and 19 females; median age: 44 years (range 14-61) subtype FAB: M0: 2; M1: 5; M2: 9; M4: 13; M5: 1). 14 patients have been conditioned with BuMel (5 M and 9 F; median age: 44 years (range 18-61) subtype FAB M1: 3; M2: 5; M4: 5; M5: 1) and 16 with BuCy-2 (6 M and 10 F; median age: 41 years (range 14-59) subtype FAB M0: 2; M1: 2; M2: 4; M4: 8). The factors of risk in the 2 groups are similar. High risk: 4 patients (2 in every group), intermediate risk: 22 (12 in BuCy-2 and 10 in BuMel) and low risk: 4 (2 in every group). The PBSC has been the source of the stem cells in all patients, and the median CD34 infused cells has been of 5,15 and 5,2×10^6/Kg in BuCy-2 and BuMel groups respective-ly. All patients have achieved a full haematological recovery. The median days to neutrophil> 1000/mm³ and platelets> 20000/mm³ have been of 14 and 12 days in the BuCy-2 and BuMel groups respectively. In the BuMel group one patient is died for mycosis (mucor) at day +27 after transplantation and one patient has developed a second leukemia a
month +36 (patient with down syndrome), in BuCy2 group one patient is died for acute hepatitis from reactivation HBV and one patient has developed a myelodisplasia a months +60 after transplantation. With median follow-up of 21 months (range 3-72 months), after autotransplant, 8 patients (57%) they are alive (7 in CR) in BuMel group; in the BuCy-2 group the median DFS and OS are 9 and 12 months respectively (Figures 1-2). The EFS projected to 45 months is 50% and 35% (Figure 3) in BuMel and BuCy-2 groups respectively, this difference is not statistically significant ($p$: 0.06, Cox F-test). In conclusion, even if the number of the patients is small, the difference in terms of DFS, OS and EFS doesn’t seem significant among the two regimes of conditioning. Is necessary a large cohort and a randomized study to confirm these preliminary data.

**PU-081**

**RITUXIMAB AND CHOP/CVP REGIMEN IN HIV NON HODGKIN LYMPHOMA PATIENTS**

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1Clinica Ematologica Fondazione IRCCS Policlinico San Matteo Pavia, Clinica Malattie Infettive Fondazione IRCCS Policlinico San Matteo, Università di Pavia Pavia; 2Clinica Malattie Infettive e tropicali Fondazione IRCCS Policlinico San Matteo- Università di Pavia Pavia; 3Istituto di Anatomia Patologica Fondazione IRCCS Policlinico San Matteo, Università di Pavia, Italy

The role of immunochemotherapy Rituximab plus CHOP or CVP regimen in B cell non Hodgkin lymphomas is well assessed but it is not the case in HIV lymphoma patients. We have treated 5 HIV patients affected by B cell non Hodgkin lymphoma according to R-CHOP regimen (Rituximab 375 mg/mq, Cyclophosphamide 750 mg/mq, Doxorubicin 50 mg/mq, Vinristine 1.4 mg/mq and Prednison 100 mg x5 days). Four patients started the treatment at onset the disease and one at the first relapse, occurred after ESHAP regimen. All patients were contemporaty treated with highly active antiretroviral therapy (HAART). The main clinical data are reported in Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Stadium</th>
<th>Therapy &amp; response</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>Male</td>
<td>44</td>
<td>NHL B omblastic High grade</td>
<td>IIB</td>
<td>5 R-CHOP CR 12 months</td>
</tr>
<tr>
<td>SV</td>
<td>Female</td>
<td>46</td>
<td>Relapsed Follicular B NHL</td>
<td>IIIA</td>
<td>4 R-CHOP CR 11 months</td>
</tr>
<tr>
<td>RR</td>
<td>Male</td>
<td>42</td>
<td>NHL follicular grade 2</td>
<td>IIA</td>
<td>6 R-CHOP CR for 5 months than 4 R-CVP: Continuing CR after 5 months</td>
</tr>
<tr>
<td>GC</td>
<td>Female</td>
<td>42*</td>
<td>NHL</td>
<td>I A</td>
<td>4 R-CHOP+ RT CR 26 months</td>
</tr>
<tr>
<td>ZD</td>
<td>Female</td>
<td>46</td>
<td>NHL</td>
<td>I A</td>
<td>4 R-CHOP continuing CR after 26 months</td>
</tr>
</tbody>
</table>

*Deceased.*

A total of 4-6 courses of chemotherapy were administered without delay every 21 days. Two patients developed severe neutropenia that required admission in hospital, however prophylactic G-CSF administration was able to overcome the problem and generally the patients well tolerated the therapy. One patient relapsed after 26 months of CR and died for the progression of lymphoma. None patient had a CD4 value less than 100/l. HAART therapy was also well tolerated along the chemotherapy without development of opportunistic infections. The TAC/PET complete remission was documented in 4 out of 5 patients and it was maintained at the follow up after 11-28 months. Our data confirm that Rituximab plus CHOP/CVP chemotherapy can be considered effective and well tolerated in HIV-B-NHL patients with achievement of prolonged CRs. These data of efficacy and feasibility are also supported by the fact that we have not documented opportunistic infections related to HIV disease.
**PU-082**

**INTERMEDIATE DOSE MELPHALAN IN ADVANCED MYELOMA**

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Six patients (mean age 68 yrs, r. 53–80) affected by multiple myeloma (2 G-kappa, 2 G-lambda, 1 A-lambda, 1 kappa) in progression after several lines of treatment (MP/Tal-Dexa/Vel-Dexa or aU/BMT/Tal-Dexa/Vel-Dexa) received i.v. infusions of melphalan at the dose of 25–30 mg/m², plus dexamethasone 40 mg/d x 4-days (mean number of courses: 2, r. 1-3). A beneficial response was verified in 4 patients (80% reduction of MC or disappearance of bone swelling in 2 cases; 50% MC reduction in 2 cases; significant pain reduction in all cases), with mean duration of 4 months (r. 3–6). Two patients were refractory. Significant myelosuppression occurred in all cases: all patients received erythrocyte transfusions, antibiotic prophylaxis and G-CSF. However, no one experienced severe adverse events and all procedures were performed as out-patient, with twice-weekly blood count controls. This therapeutic resource seems to offer significant possibility of response, although short lasting, in severely advanced patients, also if refractory or no more responsive to regimens based on conventional dose melphalan, thalidomide, bortezomib, or relapsing after aU/BMT.

**Table 1.**

<table>
<thead>
<tr>
<th>Age/dx/pr.of dx.</th>
<th>Previous treatments</th>
<th>Melphalan dose (total mg)</th>
<th>Courses</th>
<th>Response</th>
<th>Duration</th>
<th>G-CSF</th>
<th>RBC transfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 72/Gambldia/2003</td>
<td>MP/Tal-Dexa/ Vel-deza</td>
<td>40</td>
<td>2</td>
<td>30% CM reduction</td>
<td>+4</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>2 66/Gabbia/2001</td>
<td>aU/BMT/Tal-Dexa/ Vel-deza</td>
<td>50</td>
<td>3</td>
<td>Bone swelling disappearance</td>
<td>6</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>3 80/Gambldia/2000</td>
<td>MP/NOP/Tal-Dexa/ Vel-deza</td>
<td>40</td>
<td>1</td>
<td>30% CM reduction</td>
<td>+3</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>4 53/kappa/1999</td>
<td>aU/BMT/Tal-Dexa/ Vel-deza</td>
<td>50</td>
<td>2</td>
<td>80% CM reduction</td>
<td>+3</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>5 67/Gambldia/2006</td>
<td>MP/Tal-Dexa/ Vel-deza</td>
<td>50</td>
<td>1</td>
<td>Refractory</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>6 74/Gabbia/2003</td>
<td>MP/Tal-Dexa/ Vel-deza</td>
<td>30</td>
<td>1</td>
<td>Refractory</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

**PU-083**

**EVALUATION OF IN VITRO CHEMOSENSITIVITY AT FLUDARABINE IN B CELL CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS**

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Objectives. A percentage of patients affect by B cell chronic lymphocytic leukemia (B-CLL) is resistant to the treatment with fludarabine monophosphate. Aim of this study was to evaluate in vitro chemosensibility to fludarabine monophosphate in patients affect by B-CLL, before monophosphate. Aim of this study was to evaluate the patients. Percentage of cell mortality (quantified by using iodure de propidium in (20-10-5-2.5-1.25 umoli/mL); after 48 h of incubation was evaluated the cultured with decreasing doses of fludarabine monophosphate.

Results. The Resp patients showed a percentage of cellular mortality vs. of 26±5±13.5±% (range 12–30±%) vs. 4.7±% (range 0–9±%) of the N-Resp patients (p=0.00001). Thirteen of 32 patients were defined Resp patients (treated with only Fludarabine); of these 12 (92±%) obtained a Complete Remission and 1 a Partial Remission. Nineteen of 32 patients were defined N-Resp patients (treated with Fludarabine+ Cyclophosphamide); of these 9 obtained a Partial Remission and 10 were resistant at treatment. Nobody of N-Resp patients obtained a Complete Remission. Conclusions. Data reported suggest that exist a directed correlation between in vitro chemosensibility and clinical response to the treatment with Fludarabina. In agreement with the data of the literature we confirm that the added of Cyclophosphamide to the treatment allows to obtain an increase of the clinical response, affecting positively on the patients who have demonstrated resistance in vitro to the fludarabine.

**Table 1.**

<table>
<thead>
<tr>
<th>Pt.</th>
<th>UCB</th>
<th>NC dose (x10⁷/kg)</th>
<th>CD34+ cells (x10⁴/kg)</th>
<th>HLA disp.</th>
<th>LOCUS disp. (x10⁴/kg)</th>
<th>CFU tot</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MICB</td>
<td>3.2</td>
<td>3.9</td>
<td>4/6</td>
<td>HLA-A2; HLA-B50</td>
<td>3.14</td>
</tr>
<tr>
<td>2</td>
<td>DUCB1</td>
<td>1.7</td>
<td>0.5</td>
<td>4/6</td>
<td>HLA-B50</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>DUCB2</td>
<td>1.74</td>
<td>1.2</td>
<td>4/6</td>
<td>HLA-A2; HLA-B50</td>
<td>2.27</td>
</tr>
<tr>
<td>4</td>
<td>MUCB</td>
<td>2.1</td>
<td>0.9</td>
<td>5/6</td>
<td>HLA-A33</td>
<td>2.13</td>
</tr>
</tbody>
</table>

**PU-084**

**SEQUENTIAL MOLECULAR MONITORING OF CHIMERISM STATUS IN TWO PATIENTS TRANSPLANTED WITH DOUBLE UNRELATED DONOR UMBILICAL CORD BLOOD**


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Introduction. Unrelated donor umbilical cord blood transplantation (UCBT) has become a standard therapeutic option for children and adults with hematological malignancies, lacking an HLA identical donor. The nucleated cell (NC) dose is the most important factor influencing hematopoietic recovery and overall outcome following UCBT. The limited cell dose of the individual UCB units has been the major drawback to their broader clinical use. More recently, transplants with two unrelated UCB have been carried out in order to overcome the cell dose limitation of the single CB units. In the present study we report the clinical data and the chimerism status of two adult acute leukemia patients who received a double UCBT. Patients and Methods. Two 24 and a 29-year old men affected by acute lymphoid leukemia in 3rd complete remission (CR) and acute promyelocytic leukemia in 3rd molecular positive CR, were transplanted with a double UCB. The identification, number of nucleated cells, HLA disparities and CFU assays are reported in the Table 1. Sequential molecular monitoring of the chimerism status to evaluate hematopoietic engraftment at different time points was performed by STR-PCR. Patientes were conditioned using a myeloablative regimen. Results. The post-transplant molecular monitoring of patient 1 showed at day +21 a condition of triple chimerism in the bone marrow (BM) and peripheral blood (PB) with a prevalence of the MICB unit over the DUCB1 unit (78.7% vs 18%) that converted to a full donor status at days +60 and +100. At these time points, at variance from what observed at day +21, a persistent prevalence of the DUCB1 unit over the MICB one (98% vs 2%) was observed. The patient is presently alive and well without evidence of leukemia with a follow-up >210 days. In patient 2, the chimerism status at day +26 showed the persistence of a relevant amount of recipient hemopoiesis with the simultaneous presence of similar amounts of the two UCBs (recipient 15% vs DUCB2 44% vs NYCB 41%). The patient suffered a clinical relapse at day +60 and died at day +95 of his disease. Conclusions. Our data suggest that in patients receiving a double UCBT the post-transplant monitoring of the chimerism status by STR-PCR may be relevant to clarify the biological mechanisms underlying hematopoietic engraftment and in predicting the clinical outcome.

**Table 1.**

<table>
<thead>
<tr>
<th>Pt.</th>
<th>UCB</th>
<th>NC dose (x10⁷/kg)</th>
<th>CD34+ cells (x10⁴/kg)</th>
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<th>LOCUS disp. (x10⁴/kg)</th>
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<td>1</td>
<td>MICB</td>
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<td>4/6</td>
<td>HLA-A2; HLA-B50</td>
<td>3.14</td>
</tr>
<tr>
<td>2</td>
<td>DUCB1</td>
<td>1.7</td>
<td>0.5</td>
<td>4/6</td>
<td>HLA-B50</td>
<td>0.25</td>
</tr>
<tr>
<td>3</td>
<td>DUCB2</td>
<td>1.74</td>
<td>1.2</td>
<td>4/6</td>
<td>HLA-A2; HLA-B50</td>
<td>2.27</td>
</tr>
<tr>
<td>4</td>
<td>MUCB</td>
<td>2.1</td>
<td>0.9</td>
<td>5/6</td>
<td>HLA-A33</td>
<td>2.13</td>
</tr>
</tbody>
</table>
HOST DISEASE REFRACTORY TO IMMUNOSUPPRESSIVE THERAPY
UVA1 PHOTOTHERAPY AS A TREATMENT FOR SCLERODERMIC CHRONIC GRAFT VERSUS HOST DISEASE

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A 52 years old patient with kappa light chain myeloma underwent double autologous stem cell transplantation. During the second procedure, apheresis of PBSC after conditioning chemotherapy was performed. The patient had diagnosis in June 1999 and underwent chemotherapy with VAD for four cycles, obtaining a partial remission. In December 1999 he was mobilized by cyclophosphamide 7 gr/m², followed by G-CSF and was submitted to the first apheresis of PBSC at day +11 from chemotherapy harvesting 10,1×10⁶/kg CD34⁺. In January 2000 the patient underwent the first autologous transplantation, using a part of his tem cells, obtaining complete remission. The conditioning chemotherapy was melphalan 200 mg/m². Maintenance therapy with alpha-IFN was carried out. In October 2004 the disease relapsed. The patient underwent therapy with thalidomide and dexamethasone. In January 2005 the patient had b endocarditis caused by Gram+ bacteria. In March 2006 the disease progressed and therapy with Bortezomib and dexamethasone was scheduled. In May 2006 treatment with melphalan and cortisone was scheduled. In July 2006 the patient underwent a second autologous transplantation, using the last part of his stem cells. The conditioning regimen was: cyclophosphamide 60 mg/kg from day -5 to -2; Thiotepa 150 mg/m² from day -3 to -1; Busulfan 1 mg/kg from day -3 to -1. In day +1 a single dose of PEG-CSF was given. On day +11 a second apheresis of PBSC was performed, harvesting 1,5×10⁶/kg CD34⁺. The patient obtained a partial remission. In October 2006 the disease relapsed and the patient died for infectious complications. This clinical report shows that PEG-G-CSF after adequate conditioning chemotherapy allows apheresis of a sufficient number of stem cells, which can be helpful in patients needed additional mobilization after autologous transplant.

THE SEA URCHIN INSULATOR SNS-5 IMPROVES ONCORETROVIRUS VECTOR EXPRESSION IN ERYTHROID CELLS

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Introduction. Given the success of vectors such as murine gamma retroviruses (MLV) in human gene therapy trials, it is crucial to develop effective strategies to prevent silencing and position effect variegation of transgene expression and to avoid insertional activation of host oncoproteins. One widely proposed strategy to improve both efficacy and safety of gene therapy is the inclusion of chromatin insulators into gene delivery vectors. Here we report that the sea urchin chromatin insulator sns-5, cloned in flanking position in MSC oncoretroviral based vectors, was capable to reduce the influence of the surrounding chromatin on the expression of a reporter gene in mouse erythrocyt leukemia cell line (Mel). Methods. A 465bp long fragment, containing the sea urchin chromatin insulator sns-5 was cloned in both orientations into the SLTR of MGPN2 reporter vector to generate vector S (sense orientation) and vector R (reverse). Unsilnced (vector M) and insulnced vectors were transduced in Mel cells and the frequency of expression and the position effect variegation were analyzed. By chromatin immunoprecipitation assay (ChIP) we analysed the chromatin conformation of the integrated vectors and the in vivo binding of trans-acting factors to sns-5. Results. Analysis of vector expression in Mel cells showed that: (i) sns-5 increased the frequency of the transgene expression in that, in a limiting dilution
PU-089

WALDENSTRÖM MACROGLOBULINEMIA EVOLVING INTO MULTIPLE MYELOMA: A CASE REPORT

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Waldenstrom Macroglobulinemia (WM) is a lymphoproliferative disease with clinical and laboratory findings intermediate between lymphoma and myeloma. About 6% of cases may evolve into immunoblastic lymphoma; rare cases of acute lymphoblastic leukemia or immunoblastic lymphoma are reported. Some cases of WM in multiple myeloma (MM) are very unusual. We report a case of a 68 year old woman who had diagnosis of MGUS in 1998 and came to our observation, though asymptomatic, in 2002. Skeleton radiography didn’t show osteolyse; the monoclonal component (MC) was 2.07 g/dL (IgM kappa), blood counts were normal, k urinary chain (kappa) was 356 mg/L. By bone marrow aspirate lymphocytes and plasmacells were in the normal range; however lymphocytes typing by flow cytometry showed a b cell lymphoproliferative disease CDS-5, CD22+, SmIg-k+; Tc99m-sestamibi was negative. In september 2003 MGUS evolving in WM: patient is hypertensive, MC is increased (4.2 g/dL) and k urinary chain (kappa) was 356 mg/L; one start a therapy with chlorambucil at dose of 26 mg/day for 4 days/month. No improvement after 4 cycles. After another increase of MC, start a treatment with Rituximab at dose of 600 mg/day/week for a month. At that time there’s slight cut in hemoglobin (Hb) and leucocytes (WBC). After this therapy no improvement too and in november 2006 on begin therapy with thalidomide at dose of 100 mg/day and cycles of this therapy no improvement too and in november 2006 on begin treatment with Rituximab at dose of 600 mg/day/week for a month. At that time the levels of antibodies were not correlated with WM expression levels of IgG in 64% of MDS, 54% of ALL, 66% of ALL, 70% of CML, 70% of MM, 100% of CMML and 66% of IM. The levels of IgG antibodies were significantly higher in chronic myeloproliferative disorders as compared as acute leukemias. A significant level of IgM antibodies were present in 60% of MDS. Among them they were present in 75% of CML, 20% of RAEB and 25% of s-AML, 10% of de novo AML, 10% of ALL, 20% of CML, 220% of MM, 100% of CMML and 50% of IM. By contrast IgG and IgM were undetectable in healthy subjects. Regression analysis showed that the levels of antibodies were not correlated with WT1 expression levels (r=0.41). These data demonstrate that humoral immune responses against the WT1 protein could be elicited in patients with haematological malignancies. Moreover, the data suggest that strong and persistent stimulation by WT1 antigen, which usually occurs in patients with a large amount of leukemic cells is needed to generate immunoglobulin isotype class switching from IgM to IgG. Finally, although Multiple Myeloma patients present low levels of WT1 transcript in BM, they have significant amount of antibodies in the serum. These data suggest that patients affected by haematological malignancies, including Multiple Myeloma patients could be candidate for WT1 based immunotherapy.

PU-090

REDUCED INTENSITY CONDITIONING (RIC) AND BONE MARROW TRANSPLANTATION IN HIGH RISK MALIGNANCY: A SINGLE CENTRE EXPERIENCE

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In the last ten years reduced conditioning intensity (RIC) for Bone Marrow Transplantation has been more and more applied in patients at high risk for complications as a therapeutic possibility specially for those patients precluded to myeloablative conditioning regimen and without further chances of cure. Up to date several questions on the setting of RIC transplantation remain unanswered and the role of this procedure has to be still elucidated. We present our experience with 30 patients with several hematologic tumors. The status at RIC was resistance in 21 pts, progressive relapse in 2 pts and CR in 7 pts; 6 pts in CR had high risk AML with age older than 60 years ant the other one was HD in third remission. The overall mean age was 55.8 years, range 25-70 years. Conditioning consisted of Fludarabine 30 mg/m^2/die over 2 days + Ciclophamide 30 mg/Kg/die over 2 days + Thiotepa 10 mg/Kg in 11 pts with lymphoma and 1 pt with solid tumor. Thiotepa 10 mg/Kg and Fludarabine 25 mg/m^2/die over 5 days in 14 pts with AML or MDS, Thiotepa 5 mg/Kg + Fludarabine 30mg/m^2/die over 3 days and Melphalan 80mg/m^2 in 4 pts with MM. GVHD prophylaxis was done by CY-A and MTX in all pts. At a mean follow-up of 26 months 10 pts are alive, 8 of them in CR and 6 with cGVHD, and 20 pts died; the cause of death were interstitial pneumonitis in 3 pts, aGVHD in 4 pts and progression of disease in 15 pts. TRM was 23.5% overall with more than 30% in previously heavily treated patients. Alive pts are 6 with AML, 2 with HD and 2 with NHL. Five pts with AML and 3 with lymphoma alive at follow-up had CR at the time of TM0 and 2 had relapse. The conclusions are that RIC transplantation is indicated in pts with haematologic malignancies at high risk but with low tumor burden and responding phases of disease. Transplanted related mortality in patients heavily treated is high.

PU-091

DETECTION OF HUMORAL IMMUNE RESPONSES AGAINST WILMS TUMOR GENE (WT1) PRODUCT IN PATIENTS AFFECTED BY HAEMATOLOGICAL MALIGNECIES

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The Wilms tumor gene (WT1) is expressed at high levels in many types of haematological malignancies including Acute Myeloid Leukaemia (AML), Acute Lymphoid Leukaemia (ALL), Chronic Myeloid Leukaemia (CML), Ph negative Myeloproliferative Disorders (CMPD) and Multiple Myeloma. The WT1 transcript is also expressed in many types of solid tumours. Humoral immune responses against WT1 product has been described in Acute Leukemias and in Chronic Myeloid Leukemia (CML). At present, many clinical trials using WT1 peptides are ongoing. The aim of the study was to investigate the presence of a humoral response against WT1 protein in patients affected by haematological malignancies in order to explore the possibility to use an immunotherapy based on WT1 with vaccination using WT1 peptides or protein. After informed consent sera and Peripheral Blood samples were collected from 98 patients: 30 Myelodysplastic syndromes (MDS), 11 Acute Myeloid Leukemia (AML), 4 Acute Lymphoblastic leukaemia (ALL), 23 Multiple Myeloma (MM), 20 Chronic Myeloid Leukemia (CML), 6 Idiopathic Myelofibrosis (IM), 4 Chronic Myelomonocytic Leukaemia (CMML). In addition 20 healthy subjects were evaluated as control. Using dot blot technique we analyzed the presence of WT1 IgG and IgM antibodies. WT1 transcript amount was evaluated by quantitative Real Time PCR in PB samples. We detected a significant levels of IgG in 64% of MDS, 54% of AML, 66% of ALL, 70% of CML, 70% of MM, 100% of CMML and 66% of IM. The levels of IgG antibodies were significantly higher in chronic myeloproliferative disorders as compared as acute leukemias. A significant level of IgM antibodies were present in 60% of MDS. Among them they were present in 75% of CML, 22% of RAEB and 25% of s-AML, 10% of de novo AML, 10% of ALL, 20% of CML, 22% of MM, 100% of CMML and 50% of IM. By contrast IgG and IgM were undetectable in healthy subjects. Regression analysis showed that the levels of antibodies were not correlated with WT1 expression levels (r=0.41). These data demonstrate that humoral immune responses against the WT1 protein could be elicited in patients with haematological malignancies. Moreover, the data suggest that strong and persistent stimulation by WT1 antigen, which usually occurs in patients with a large amount of leukemic cells is needed to generate immunoglobulin isotype class switching from IgM to IgG. Finally, although Multiple Myeloma patients present low levels of WT1 transcript in BM, they have significant amount of antibodies in the serum. These data suggest that patients affected by haematological malignancies, including Multiple Myeloma patients could be candidate for WT1 based immunotherapy.

PU-092

SPONTANEOUS SPLENIC RUPTURE IN DIFFUSE LARGE B-CELL LYMPHOMA: A CASE REPORT

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Purpose. Spontaneous rupture of the spleen is an uncommon, dramatic abdominal emergency that requires immediate diagnosis and prompt...
surgical treatment to ensure the patient’s survival. Spleenic rupture are rare in hematological malignancies despite frequent involvement of the spleen. We report a case of spontaneous splenic rupture caused by infiltration of B-large cell lymphoma variant T-cell rich Methods. Observational case report. Results. 40-year old man presented to the Emergency Department with a day old history of acute left upper abdominal pain, nausea and vomiting. The patient underwent an emergency a ultrasonography examen and successful a contrast enhanced CT scan of the abdomen/pelvis which showed an markedly abnormal spleen, inhomogeneous and enlarged, approximately 20×11 cm with fluid in the inferi or aspect of the mesenteric fat, with free fluid in the abdomen and enlarged retroperitoneal lymph nodes. Since there was no history of trauma and the CT did not look like a traumatic splenic laceration. The biochemistry test evidence relevant anemia (Hb 7 g/dL), higher level of LDH and beta 2 microglobulin and hypogammaglobulinemia; patient underwent a splenectomy. The histological exam evidence the splenic involvement by diffuse large B-cell lymphoma variant T-cell rich; The PET study was following performed with evidence the activity in multiple thoracic, abdomen and retroperitoneal lymph nodes; bone marrow biopsy showed the absence of involvement. Successful The patients had been demonstrated. The splenic rupture occurred before the start of antitumoral chemotherapy, which may have released lytic and vasoactive enzymes due to chemotherapy-induced cell necrosis. Emergency splenectomy represents the only feasible treatment for splenic rupture.

PU-093

EFFECTS OF RITUXIMAB ON THE MOBILIZATION AND ENGRAFTMENT OF AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS IN DIFFUSE-LARGE B-CELL LYMPHOMA


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Treatment with rituximab is widely used for diffuse large B-cell non Hodgkin’s lymphomas (DLBCL). However, its effects on peripheral blood stem cell mobilization are not completely known. We retrospectively evaluated 28 patients with DLBCL responding to first-line chemotherapy (CHOP or R-CHOP), but failing to achieve complete remission (CR). In this group we compared the mobilization characteristics, engraftment kinetics and transplant related toxicity of 15 patients receiving (R)-CHOP and 13 not receiving rituximab (CHOP) as a first-line treatment before autologous peripheral blood stem cells transplantation (PBSCT). Patients mean age was 57 years (range 17-80); 28 patients (82.1%) had stage III-IV disease. 17 patients (56.7%) had bone marrow involvement; systemic B symptoms were present in 16 patients (57.1%). Mobilization chemotherapy consisted of a high dose cytarabine containing regimen (DHAP) in all patients. The median CD34+ cells collected was 4.7×10^6/kg in patients receiving rituximab vs 6.8×10^6/kg CD34+ cells (p=n.s.) in the non rituximab treated group. Failure to mobilize, defined as failure to reach a circulating CD34+ cell count of 10/μl, occurred in 2 patients (13.5%) in the rituximab group and 3 (23.1%) in the non rituximab group. All patients were transplanted using myeloablative chemotherapy conditioning regimen (BEAM); Comparison of the two groups showed no statistical significant difference between median days to absolute neutrophil >0.5×10^9/L and platelet >20×10^9/L counts after PBSC, and no differences in incidence and severity of infections, days of fever or duration of antibiotic treatment between groups. In conclusion, our data provide evidence that rituximab in association with the CHOP regimen as first line treatment in DLBCL has no adverse effect on the mobilization and engraftment of PBSC. Rituximab is employed in the first-line treatment for newly diagnosed DLBCL patients. Further studies are warranted in larger populations to determine the impact of rituximab on collection, engraftment and survival.

PU-094

AN OLD MALE WITH SIMULTANEOUS PRESENTATION OF HODGKIN DISEASE AND NON HODGKIN LYMPHOMA: CASE REPORT AND LITERATURE REVIEW

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While Hodgkin’s Lymphoma (HL) and the non Hodgkin’s Lymphomas (NLH) have long been regarded as distinct disease entities, recent observations suggest a closer association. The analysis of cases in which both diseases are present in the same anatomic site (composite lymphomas), or in separate sites (simultaneous or sequential HL and NLH), indicates that this phenomenon occurs more frequently than would be expected by chance alone. The most common form of composite lymphoma is Nodular Lymphocyte-Predominant Hodgkin’s Lymphoma (NLPHL) associated with Large Cell Lymphoma (LCL) of B cell phenotype. This finding is consistent with a B cell origin for the abnormal cells in NLPHD, suggesting that LCL represents a form of histologic progression. We report the case of a 85 year old male patient with coexistent classic variant HL and DLBCL, a less common association. He was admitted in our institution with persistent fever, weight loss, one right inguinal lymph node sized 1.5 cm and splenomegaly. Abdomen ultrasounds showed splenic enlargement with hypoechochogenic nodules. Blood cell count revealed a mild anemia; LDH levels were normal, beta-2 microglobulin levels were increased. A total body Computed Tomography (CT) showed a mass in left inferior lobe of the right lung (4,5 cm large), confirming splenomegaly with splenic hyperdense nodules and pathologic celiac lymph nodes. We performed a CT-guided trans-panreal liver biopsy: the histology was consistent with Large B cells NHL (LCA; CD 20+; CD 5-; CD 30-); on the contrary the splenic biopsy showed the presence of large stembergoid cells CD 30+ e CD 15+ CD 20–, CD 45–, CD 25–; we collected was 4.7 g/dL, higher level of LDH levels were normal, beta-2 microglobulin in the non rituximab group. All patients were transplanted using myeloablation.

PU-095

URETHRAL LYMPHOMAS. A RARE BUT POSSIBLE DISEASE LOCALIZATION. THINKING ABOUT THERAPEUTICAL OPTIONS.


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Introduction. Extranodal malignant lymphomas rarely affects the urinary tract and the urethra being this last one, the most uncommon site of origin. Usually the most frequent ones are low grade non Hodgkin lymphomas. We present a case of primary high grade non Hodgkin lymphoma arising from the female urethra. Case Summary. A 78 years old woman was admitted in hospital presenting anuria and abdominal pain. She had a history of important weight loss in the previous months, but there wasn’t a history of recurrent urinary infections. Cystourethroscopy demonstrated a neoplasm interesting external urethral meatus. A trans urethral biopsy was performed and a large B cell non Hodgkin lymphoma diagnosis was posed. Immunohistochemical stains for cytocheratin and CD20 were negative, while CD20 and LCA were positive. A CT scan demonstrated a mass infiltrating the lowest site of the bladder and the urethral wall. Hematological and biochemical examinations were normal; chest radiography and CT scan of upper abdomen and chest were positive for the presence of a few lymphoadenopathies. Bone.
1 to 1.5% of NHL arise from female genital or urinary tract. However, these tumors are rarely the site of origin of this type of neoplasia; in fact only 1 to 1.5% of NHL arise from female genital organ. They are most commonly localized in the ovaries (49%), uterus (29%) and fallopian tubes (11%) but only 0.6% in the uterine cervix. We present a case of primary high grade non Hodgkin lymphoma arising from the lower female genital tract.

**Case Summary.** A 25-year-old white female presented to her primary care physician because of persistent back pain without systemic symptoms. A MRI of the abdomen and pelvis revealed an uterine cervix mass with a diameter of 7 cm. On physical examination, a large cervical mass was easily palpable and a focal vaginal mucosa lesion and left parametrial involvement were also noted. Multiple biopsies of cervical and vaginal mucosa revealed diffuse infiltration by atypical lymphoid cells. Immunophenotyping was compatible with diagnosis of diffuse large B-cell lymphoma. Clinical staging work-up, including a total body CT scan and a bone marrow biopsy was indicated to stage IVs disease. The patient underwent 5 previous therapeutic lines. The time from diagnosis to the beginning of the PAD cycles was 120 months and 2 months from the latest treatment. The patient was critical for heavy anaemia, bone pain and astenia.

**Discussion.** Extramedul lary lymphomas arising from the female genital tract are very rare. Because of up to 2/3 of these tumors presents with subepithelial mass without epithelial abnormality, a deep incisional or excisional biopsy is recommended when the initial biopsy is non diagnostic. For this reason a Pap smear test is rarely useful, leading to a false negative cytologic smear. Because cervical NHL is an uncommon malignancy, the optimal treatment has not been established. The available treatment strategies for their management are chemotherapy alone, radiotherapy alone, or radiotherapy combined with either chemotherapy or surgery. As these tumors are extremely responsive to chemotherapy, taking into account the effectiveness of adding rituximab to the CHOP-like polichemotherapeutic approach, it could be opportune, mainly in young women, the use of chemoimmunotherapy (e.g. R-CHOP) alone, avoiding radiotherapy also in I-IE stages and whenever possible, in order to preserve fertility without compromising possibility to get healing.

**PU-096**

**FEMALE GENITAL NON HODGKIN’S LYMPHOMA: HEALING WITHOUT SURGERY**

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**Introduction.** Non Hodgkin’s Lymphomas frequently affect the uterine corpus, cervix and vagina in cases of advanced disease. However these organs are rarely the site of origin of this type of neoplasia; in fact only 1 to 1.5% of NHL arise from female genital organ. They are most commonly localized in the ovaries (49%), uterus (29%) and fallopian tubes (11%) but only 0.6% in the uterine cervix. We present a case of primary high grade non Hodgkin lymphoma arising from the lower female genital tract. A MRI of the abdomen and pelvis revealed an uterine cervical mass with a diameter of 7 cm. On physical examination, a large cervical mass was easily palpable and a focal vaginal mucosa lesion and left parametrial involvement were also noted. Multiple biopsies of cervical and vaginal mucosa revealed diffuse infiltration by atypical lymphoid cells. Immunophenotyping was compatible with diagnosis of diffuse large B-cell lymphoma. Clinical staging work-up, including a total body CT scan and a bone marrow biopsy was indicated to stage IVs disease. The patient underwent 5 previous therapeutic lines. The time from diagnosis to the beginning of the PAD cycles was 120 months and 2 months from the latest treatment. The patient was critical for heavy anaemia, bone pain and astenia.

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**PU-097**

**USE OF BORTEZOMIB IN RECURRENT AND REFRACTORY MULTIPLE MYELOMA AND IMPAIRED RENAL FUNCTION**

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Multiple myeloma, a hematologic neoplasm characterized by the monoclonal proliferation of bone marrow plasmacells, presents renal failure in approximately 30% of patients at diagnosis and in 50% of those with an advanced form of the disease. The severity of renal impairment significantly affects the prognosis of patients with multiple myeloma and it is associated with decreased response; shorter survival and early mortality. Treatment options such as Melphalan-based chemotherapy and high dose chemotherapy with autologous stem cell transplantation need dose adjustment to avoid serious toxicities. In recent years, new therapeutic options, such as Thalidomide, Lenalidomide and Bortezomib have been introduced for the treatment of multiple myeloma with improvement outcomes of the patient. Thalidomide has proven to be efficacious in patients with renal impairment and relapse of myeloma refractory to chemotherapy. Lenalidomide has been shown to undergo substantial elimination through the kidneys and no clinical data are known about its use in patients with renal failure. A subanalysis of patients with renal impairment treated with Bortezomib in the SUMMIT and CREST trials showed no difference on response rates, treatment discontinuation and toxicities compared to overall study population. We have analyzed data of 4 multiple myeloma patients with renal impairment function out of 25 that received chemotherapy with Bortezomib 1.3 mg/mg day 1, 1.4, 8, 11, Lomosoal Doxorubicin 80 mg/mq day 1 and Dexamethasone 40 mg/day 1-4 (PAD) for relapsed or refractory disease.

**Case 1.** The patient, who was 66 years old, affected by multiple myeloma IgA k and CS IIIA at diagnosis received cycles PAD for relapsed of disease. The patient underwent 5 previous therapeutic lines. The time from diagnosis to the beginning of the PAD cycles was 120 months and 2 months from the latest treatment. The patient was critical for heavy anaemia, bone pain and astenia. The patient completed 8 cycles obtaining renal function improvement and a partial response of disease. We observed grade II hematological toxicity: leucopenia. The Follow-up was at its 12th month from the disease relapse. A 56-year-old male with multiple myeloma IgG k, CS IIIA was treated for relapse disease. Time from diagnosis and time from latest therapy was 104 and 10 months respectively. The patient received previously 4 therapeutic lines. The value of Cr Cl was 26 mL/min, serum creatinine 2.0 mg/dL, GFR 33,8 mL/min/1.73 m2, serum IgG level 3.7 g/L. The patient completed 8 planned cycles. After 2 cycles, because of grade III gastrointestinal toxicity (diarrhea), the dose of Bortezomib was reduced to 0.7 mg/mq. The patient obtained a Partial Remission and the restore of renal function improved (Cr Cl was 50 mL/min, serum creatinine 2.5 mg/dL, Glomerular Filtration Rate 27,6 mL/min/1.73 m2 (n.v.>60) serum IgAk level 3.9 g/L. He completed 8 cycles obtaining renal function improvement and a partial response of disease. We observed grade II hematological toxicity: leucopenia. The Follow-up was at its 12th month from the disease relapse. A 66-year-old female with diagnosis of refractory multiple myeloma, light chain K, CS IIIB, received cycle PAD 7 months from diagnosis and after therapy with Thalidomide+ Dexamethasone and cycles DAV (Vincristina, Adriamycin and Dexamethasone). The patient’s Cr Cl was 15 mL/min, serum creatinine 4.5 mg/dL, GFR 11,2 mL/min/1.73 m2. The clinical condition of patient was critical for heavy anaemia, bone pain and astenia. The patient completed 8 PAD cycles. We observed grade II hematological toxicity: thrombocytopenia and leukopenia. The patient had grade II renal toxicity: serum creatinine 4.0 mg/dL, GFR 11,7 mL/min/1.73 m2. The patient completed 4 PAD cycles with a 30% reduction of monoclonal serum level of creatinine of 3.0 mg/dl. Grade II neurological toxicity was detected. The renal function improved (Cr Cl 25 mL/min, Serum creatinine level 3.0 mg/dl). The patient obtained a partial response with bone marrow plasmacellular infiltration same to 30%. Case 4. A 70 years old female with diagnosis of refractory multiple myeloma IgGk CS IIIIB treated with PAD cycles after 6 months from diagnosis and after therapy with Thalidomide plus Dexamethasone and DAV 3 cycles. The patient’s Cr Cl was 18 mL/min, serum creatinine 4.0 mg/dL, GFR 11,7 mL/min/1.73 m2. The patient completed 4 PAD cycles with a 30% reduction of monoclonal serum level of creatinine of 3.0 mg/dl. Grade II neurological toxicity sensitive the patient experiment-ed. In conclusion we observed a response to the treatment in all patients with acceptable toxicities and improvement of renal function. Our analysis confirm that Bortezomib therapy is a well-tolerated effective option in that subgroup of MM patients with renal failure.
In an ongoing phase II study we are treating unselected stage 3 myeloma’s patients, as upfront therapy, with Bortezomib and Desametasone in the context of a sequential treatment which include thalidomide and single autologous transplantation in patients younger than 70 years. Aims of the study were the evaluation of efficacy, as primary response, and toxicity of Bortezomib in previously untreated patients. The inclusion criteria were a diagnosis of multiple myeloma, stage 3 according to Durie and Salmon, unrespective of A or B. The only exclusion criteria was the documented presence of a previous neuropathy and the positivity of HCV, HIV and HBV or major organ disfunction and previous deep thrombotic events. Other minor aspects were the feasibility of the therapy and incidence of infections. The therapeutic program consisted of Bortezomib 1.3 mg/sqm days 1, 4, 8, 11, every 21 days over 4 courses, Desametasone 40mg over 4 days every 15 days over 4 months; thalidomide was started after the completion of Bortezomib program at 100 mg/day over 4 months. Autologous bone marrow transplantation with PBSC was planned in patients with age up to 70 years following CD34 mobilization by CTX 5 gr/sqm. Up to May 2007 8 pts of mean age 65 (49-77) completed the therapeutic phase inclusive of Bortezomib and are valuable for response and toxicity. The re-evaluation of bone marrow at the end of Bortezomib showed less than 5% of plasma cells in 6/8 pts (CR) and less than 10% in 2 pts (PR), the clinical response based on the disappearance or dramatic reduction of bone pain was achieved early after the first course of Bortezomib. Three patients showed peripheral grade II neuropathy which improved within 1 or 2 months following the end of therapy. The haematological toxicity was mild and transient. In a concurrent analysis in 12 pts treated by Bortezomib, same schedule, following relapse to previous therapy we observed a response in 4/12 pts. In conclusion, our preliminary report on the treatment by Bortezomib, as up-front therapy in patients with advanced MM, shows a very good efficacy in inducing remission with an acceptable toxicity.
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